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Bringing men to the Moon

"TODAY scientific technology has reached the point where man has liberated nuclear energy and sent men to the Moon". Familiar, indeed trite, words that have been repeated a hundred times and which begin the preface to the handbook of the Third International Conference on the Unity of the Sciences to be held in London next month. But in this case there is an unintentional pun in it, for Moon is also the name of the man behind it all—Rev. Sun Myung Moon. And Mr Moon is an interesting character whose organisation seems to have a magic touch at compiling international gatherings stiff with Nobel Laureates.

The conference is sponsored by the International Cultural Foundation, founded in Japan in 1968 by Mr Moon, a South Korean. Its aims are "to inspire and foster the emerging world culture and civilisation", and to achieve this it lists prospective activities which would hardly be out of place at UNESCO—except that apparently the communist half of the world is not welcome.

The first conference was in New York in 1971 and twenty attended to hear Mr Edward Haskell propound his methods for eliminating "many defects produced as a result of the fragmentation of sciences as well as the breakup of science and morals . . . by viewing the scientific disciplines from a systematic viewpoint". "We had to listen to some extraordinary ideas on unification by way of Mendeleev's periodic table", said one participant. Another was horrified that whilst the scientists thought it silly, the philosophers present all took it very seriously.

Mr Haskell's influence seems to have waned between the first conference and the second, which was held in Tokyo last year with 60 participants discussing 'Modern Science and Moral Values'. Again there was international participation and for the second time there was plenty of money to fly the "deeply concerned scholars" to Japan. There were reply paid telegrams for those who didn't go, to send greetings for later reproduction as "messages of congratulation". Nobel Prize winners were carefully cultivated and identified as such whenever possible. And Mr Moon addressed the assembled gathering in very general terms.

Amongst the hand-outs to those who attended was a book *Unification Thought*. This book, written by an unnamed Korean, is published by the Unification Thought Institute of New York and is based on the Unification Principle of Mr Moon. In the second paragraph of the Preface we are told that "communism, which is a pseudo-value system, is eroding the Free World both in public and in secret". To counter this, Mr Moon's theistic ideology is described, with emphasis

on Creation by God and the action of Divine Providence. The sweep of Mr Moon's thought is enormous and on the whole harmless. The Unification Principle "disregards the theory of evolution". The Unification Principle makes logical positivism "absurd", because of the "family Four Position Base". The "purpose" of the electron is to preserve atomic structure through revolving around the proton but also to bring joy to man indirectly. And so on. On values—the forthcoming conference is on absolute values—the writer concludes "there is no other way to find the true view of value than to perceive exactly what God's purpose for creation is". And the last chapter, on "Theory of History", begins to give us some idea what this purpose is.

History is, amongst other things, the history of re-creation and is marching towards a goal. Numerology then takes over.

First it is the number three. Everything reaches completion through three stages of growth. Three sons of Adam, three sons of Noah, three temptations of Christ and so on. Recently three stages of religious Renaissance, the first, Luther and Calvin; the second 17th and 18th century Pietism, Quakerism and Methodism; the third will develop soon on a world-wide scale when all religions and thoughts will be completely unified.

Then there is the number six. Six days of creation; six centuries of turmoil before Christ; six centuries before the appearance of the Third Adam. And the start of this last period? When the Pope became prisoner at Avignon, namely 1309. So the Third Adam should be around right now.

Next comes the number four in forty days, forty years, four hundred years.

Then, the law of false preceding true with Stalin as a false Messiah after whom we can "feel the approach of the Second Advent of the Messiah".

And finally the Law of Parallel Providence, comparing 1930 years of Old Testament development with 1930 years of development after Christ, again points clearly to an impending "Second Advent of the Messiah". So the Unification Principle exists to bring religion and science together in a unified culture and start a new cultural age under, presumably, a Third Adam or Second Messiah. Without the communists, of course.

Not that it is all theoretical. Mr Moon now has a following of a couple of million the world over and is at present crusading vigorously in the United States.





Large numbers of 'Moon children' have been imported from Japan and Korea and have affronted the American public by selling candles on street corners and showering New York and Washington with posters and pamphlets, thereby violating their visa status and breaking anti-litter laws. And large numbers of young Americans have taken it all seriously (*Time*, September 30). In the summer, Moon children were to be seen praying publicly in Washington for President Nixon. Just before a Madison Square Gardens appearance they ensured that Mr Moon had a large gathering of personal guests at a banquet at the Waldorf Astoria by camping down in United Nations missions to urge attendance on the diplomats.

The money for all this comes partly from street corner sales, but Mr Moon also owns several Korean businesses, making, amongst other things, ginseng tea and guns. The industries are given to him, it is said, by businessmen converted to the cause.

It is not clear that those invited to the Third Conference are aware of the background to the organisation. The initial brochure speaks of the purpose of the conference as discussion of the role of science in relation to problems of value and a possible way of setting up a universal standard of value for all mankind. It mentions Sun Myung Moon (without the Rev.) as the inspired founder. It lists British academic advisors, although not all on the list were very pleased to have their name used in this way. But it does not say that the fundamental philosophy is anti-communist, nor that the utterances of Mr Moon are such as to leave his followers with the impression that he himself is the Third Adam or Second Messiah.

The second round of publicity contains an extensive list of speakers—62 in all—and an even more extensive list of "commentators and discussants". No less than twenty-five of those attending have the words Nobel Laureate after their name. But none of the 150 listed comes from a communist country, nor even India, whereas three come from the Republic of China (Taiwan) and five from Korea (South Korea). A random check revealed that not everyone listed had ever had the intention of going to the conference.

The conference is being organised from an office in London. Mr Moon's book *Divine Principle* occupied pride of place on the mantelpiece. Questions were answered with charm and courtesy but generally in a rather hazy way; it could hardly be said that the philosophy of the organisation came through as anything other than the most rapid of generalisations about all sciences being one, science giving us the answers to help organise society, it not being desirable to use science for destruction and there being a spiritual dimension to science.

But have you tried to get representatives from the communist half of the world? Well it is not possible to invite people from all countries every year.

Is Mr Haskell, who played such a central role two years ago, coming this year? We aren't sure of that.

Who is this Mr Moon? (His name had modestly not been mentioned in our discussion before this point.) He is a Korean philosopher who inspired these conferences.

Is he coming? We don't know.

There is an empty slot on the packed programme for a Guest Address; who will give this? We shall be looking for someone soon.

For some, of course, the aims of this conference and its background are entirely in accord with their philosophy and they can hardly be criticised for giving it their serious attention. And some who have been dissatisfied with the previous conferences have resolved to give it another try, in order to meet people, or in the hope that the foundation has tightened up its academic credentials. But many others seem to be in almost complete ignorance of the organisation behind it and unaware that their presence may be used to confer some degree of respectability on something with which they would have little sympathy.

Certainly the names of those expected to come should inspire some confidence that the operation will not be an entirely futile affair—provided they show up. But there are lingering doubts that everything is still not quite explicit, and there is an uncomfortable amount of using of names and Nobel Prizes as a means of generating momentum.

Never is an eminent scientist happier than when jetting around the world to conferences at which his eminence in one field permits him to lecture unchecked in another. One actually expressed the view that he would rather the conference had been elsewhere than in London to give him the chance for a trip. The foundation seems to have been successful in spotting two weak points of scientists—their Boeing complex and their propensity to amateur philosophising.

And a check with Mr Moon's Washington office reveals that he is indeed planning to be in London for the conference.

A hundred years ago



THE study of "seaweeds" is probably affected as much by the general public as that of fish; and whether or not the great mass of people who visit the Brighton Aquarium and other similar resorts really go there with any idea of becoming more intimately acquainted with the wonders of the deep, there is no doubt that the exhibition of varieties of ocean plants would be as popular as that of fish. A seaweed growing in water is very different from seaweed cast up on the shore, and a careful selection and arrangement of specimens would greatly enhance the interest of the tanks, while at the same time their presence would prove beneficial to the fish. We recommend the hint to the notice of the authorities of the Brighton, Crystal Palace, and Southport Aquariums.

From *Nature*, 10, 530, October 29, 1874.

THE most notable feature of the current outbreak of rabies in Europe is the involvement of wildlife. When the disease was eliminated from Britain and certain other European countries at the turn of the century, rabies had been maintained in the domestic dog. Now it is the fox population which acts as a reservoir of infection.

Whatever the reason, this new factor makes the present outbreak difficult to stem. As Britain's foxes are now more numerous than ever before, everything possible must be done to keep the disease away from them.

Rabies could reach Britain in two ways: a domestic animal incubating the disease might be landed illegally or an infected wild creature could make its way here under its own steam. The former is much more likely but although we can discount tales of rabid foxes attempting the channel swim, the second should not be ignored.

Bats can harbour rabies and certain species are known to arrive in Britain from the continent (the common long-eared bat, for example, migrates from Scandinavia to Yorkshire). Surprisingly, although rats are susceptible to rabies and carry other diseases from country to country on board ship, for some reason they have not yet brought rabies here. But as the distance between endemic areas of wildlife rabies and Britain diminishes, the likelihood of infection reaching us in these ways, however slight, must increase. And apart from greater vigilance against any animal stowaways, there is little that can be done to prevent it.

Illegal importation is another matter. According to a recent survey by the Ministry of Agriculture, Fisheries and Food (MAFF), 110 dogs, cats and other animals were landed illegally last year at the Kent ports alone. How many escaped detection is, of course, anyone's guess. Each of these animals might have been infected with rabies (some had been adopted only hours before the crossing) and transmitted the disease to pets and wildlife here. Although the report calls for greater deterrents against would-be smugglers, the greatest hope of keeping rabies out of Britain seems to lie in alerting the public to the dangers of the disease and in explaining why quarantine procedures are needed to prevent its entry.

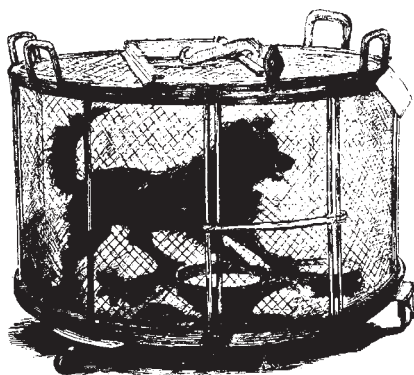
And the public do need education; Britain has been free from rabies for several generations now and many people seem to think of it as a "disease of mad dogs". They have forgotten that rabies is almost always fatal in man once the symptoms appear—only two cures have ever been recorded—and so hideous are the agonies of the victim that euthanasia was once the common 'remedy'.

Nor are they aware of the regulations

governing the importation of any animals they may acquire while on holiday. It is illegal to land an animal in Britain without an import licence and that is only given to owners who have made arrangements for six months' quarantine. Landing is held to have taken place before customs examination

Mad dogs and Englishmen

The present epidemic of rabies in Europe is still spreading westwards and has now reached northern France; it could enter Britain. John Wilson has been looking at the risks to this country.



Caged and inoculated dog, 1884

so even those people who declare their pet have committed an offence.

Official action in Kent last year prevented the entry of 33 animals but 10 evaded the port controls completely and were only detected inland (or on their way out of the country again). In all the other cases, it was the owners themselves who took action that prevented the animals from entering—and thus showed their ignorance of the law.

Customs officers reckoned that about 20 animals had been deliberately concealed. Perhaps the owners did so to avoid paying quarantine fee (about £120 for a dog) or perhaps from a misplaced sympathy of animals in quarantine. In either case, the fines handed out to the offenders (average £40) were hardly a deterrent. But under the Rabies Act (1974) fines have now been increased—£200 or more in some cases—and the local authority can have the animal summarily destroyed.

But if the worst comes to the worst how would Britain react to an outbreak? If pets or farm animals evaded quarantine there is some chance that the animal would be discovered before the disease had been transmitted to wildlife and to foxes in particular. That would be bad enough: family pets destroyed, valuable animals lost and

people subjected to pain and anxiety. But if the disease did spread to the fox population things would be grave indeed. Continental workers have shown that rabies will still survive amongst foxes until their numbers are reduced to about 0.5 individuals per square kilometre. At this point the chances of a rabid fox infecting one of its fellows before it dies begin to peter out. Following the example of other European countries, the government would adopt a 'killer' policy, however unpopular it might be with some environmentalists.

With the exception of Denmark and possibly Belgium, this 'killer' policy has not met with much success. In France the disease is advancing along a front nearly 800 km long at the rate of roughly 30 km each year. To halt it would require a high degree of control over the fox numbers in a belt about 60 km deep—a prodigious effort. Britain is isolated, however, and the disease could be eliminated before it had taken a firm hold.

Plans are already prepared. Scientists from MAFF calculate that an area of about 12 miles radius around an outbreak of rabies would need to be virtually cleared of foxes. But before the hunting fraternity reach for their stirrups they should know that their activities would not be welcome for they could drive infected animals out of the area. An intensive poisoning campaign seems best.

Not everyone believes that the introduction of rabies to this country is inevitable but it is perhaps just as well that a new vaccine will soon be ready to treat any British victims of the disease. Developed at the Wistar Institute and produced at the Mérieux Institute in France the new killed virus vaccine is prepared from cultures of WI38 human tissue culture. Unlike vaccines now in use, such as the Duck Embryo Vaccine, it contains virus antigens and very little else—rather like the Salk polio vaccine. This should ensure that the incidence of side effects to vaccination will be greatly reduced.

Although trials of the WI38 vaccine are not yet complete the initial results are very promising. Even one injection quickly produces a very high titre of antibodies. The vaccine is expensive compared with conventional rabies vaccines but work is being done at the Clinical Research Centre, Harrow, to improve the dose regimen.

If the new vaccine passes its clinical trials only one painless vaccination in the arm would be necessary rather than the traditional 14 or so painful abdominal vaccinations now required. But no matter what improvements in treatment are on the way they are no excuse to take liberties with such a distressing and expensive disease. □

international news

Nor before time, the Nobel Prize for Physics has been awarded for work in radioastronomy. Given that at last the prize was to go in such a direction, it could hardly have been awarded to anyone other than Sir Martin Ryle, who pioneered the technique of aperture synthesis (cited by the Nobel committee) which produced a revolution in radio astronomy techniques.

The long wait for Nobel recognition for any kind of astronomy (the award to Hannes Alfvén a couple of years ago was not strictly for his work as an astronomer) has encouraged various interesting rumours. Was it true, the more irreverent young astronomers wondered, that Alfred Nobel's wife had at one time had a liaison with an astronomer, as a result of which the inventor of dynamite and benefactor had excluded astronomers from the list of those eligible for an award? Alas for those who savour such gossip it now seems that those rumours can be dashed. But never mind, the significance of Ryle's work has itself caused enough confusion among the non-scientific press to enliven many an astronomical coffee break.

Perhaps the reaction of the *Wall Street Journal* best sums up the predicament of editors called upon to explain aperture synthesis for a lay readership: "Ryle refined the use of telescopes", it said on its front page, and left it at that. But the technique, like many great advances, is not so difficult to comprehend in retrospect—it is the ability to think of it in the first place which singles Ryle out for special mention.

The synthesis implied by the name of the technique involves using an array of relatively small radio telescopes to mimic some of the properties of a much larger instrument. The latest of Ryle's brainchildren is the 5-km instrument near Cambridge; there, a set of eight telescopes placed along a railway track is used to build up a picture of radio sources as they would be 'seen' by a single antenna 5 km in diameter.

This can only be achieved by making observations of the same source over hours, days and even longer. The 5-km 'aperture' is built up by the motion of the elemental antennas as the Earth rotates, and by judicious adjustment of their positions relative to one another along the railway track. You can synthesise most of the aperture for some of the time, and some

Astronomers share Nobel Prize

This year's Nobel Prize for Physics was awarded to two astronomers, Professor Sir Martin Ryle (opposite) and Professor Antony Hewish (below).



of the aperture for most of the time; you can't synthesise all of the aperture for all of the time, but the result is that with a computer it is possible to provide a map of the average intensity of the source as viewed by a non-existent 5-km dish.

So it is no surprise that Ryle's work has been recognised in this way. He already has just about every honour available to a British scientist, having been elected a Fellow of the Royal Society in 1952, appointed to the first chair of radioastronomy in the University of Cambridge in 1959 and knighted in 1966; he became Astronomer Royal in 1972. Not least among his many achievements are his teaching skills, which have probably contributed as much as his scientific ability to making the Cambridge radioastronomy group one of the best in the world.

JOHN GRIBBIN

THE communication from Hewish and his students which reached the offices of *Nature* on February 9, 1968 will surely stand as one of the classic contributions to science in this century. Even to those of us accustomed to a succession of remarkable discoveries

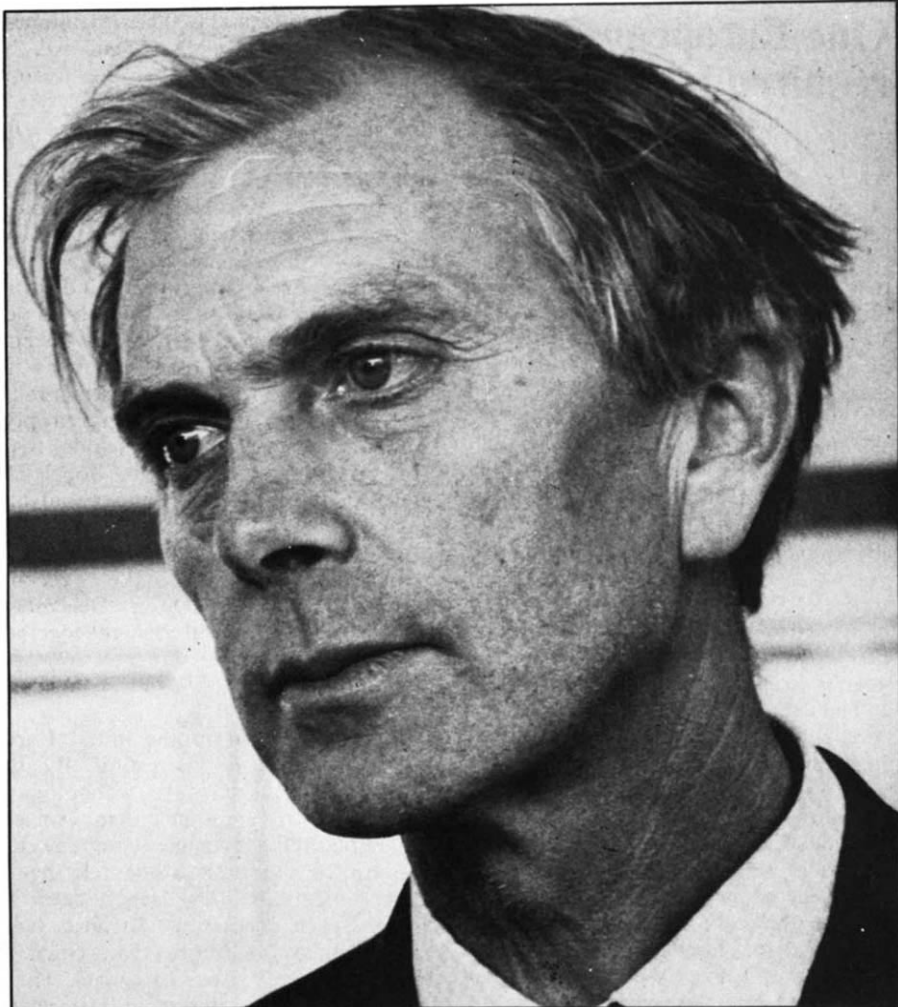
by radioastronomers, the news that there were objects in the Universe emitting radio waves in pulses, regularly spaced with intervals of about a second, strained credulity. A few days before this paper appeared in *Nature*, Fred Hoyle told me during a meeting in London that Hewish had discovered this new kind of radio source. My instant reaction was one of disbelief, an instinctive feeling that nothing in the Universe could behave like this and that his system must have been confused by the all too familiar problem of terrestrial interference. On the contrary the discovery was absolute and, in a sense which is rare in science today, the unique property of Hewish and his colleagues.

Why did Hewish find these signals when the rest of us with far more expensive and complicated radio telescopes missed them completely until we knew where and how to search, when they became obvious immediately? Hewish was studying the phenomenon of interplanetary scintillation. The effect, which he had discovered in 1964, occurs when the radio waves from a small diameter source such as a distant quasar are diffracted as they traverse the plasma clouds in interplanetary space. This induces a fluctuation in intensity with a period typically of about a second on otherwise steady signals. The study of this phenomenon was proving to be extremely useful both for the investigation of the solar corona and also for the measurement of the angular diameter of the distant radio sources. One of the last acts of the Department of Industrial and Scientific Research before it was disbanded in 1965 was to award Hewish a grant of about £17,000 to build a special aerial array which he had designed specifically for these investigations. There were at least three critical features of this concept which predestined that Hewish would discover objects in the Universe for which he was not searching. The aerial was very large, consisting of over 2,000 dipoles it covered 4.5 acres, and therefore had a far greater collecting area than any of the synthesis aerials at Cambridge, or of the steerable radio telescopes elsewhere. The receiver and pen recording system was designed with time constants to record fluctuating signals with periods of the order of a second. Finally, whereas radio astronomers all over the world were straining to work at shorter

wavelengths in order to achieve more resolution with reasonably sized aerials, Hewish remained interested in the metre waveband where the plasma effects are more pronounced than at shorter wavelengths.

In the event, the DSIR grant turned out to be one of the most cost-effective in scientific history. Less than two months after recording began, Hewish's student Miss Jocelyn Bell noted the recurrent pulses on the recording charts around local midnight when the normal scintillation phenomenon is inconspicuous. The drama of the succeeding months as Hewish established the extraterrestrial and stellar nature of the signals was hidden from the world until there could be nothing but astonishment at the final outcome. Six years later with 120 pulsars under study there seems little doubt that the signals have their origin in neutron stars, the end of a supernova explosion—a possibility suggested by Hewish in the initial publication. In these events the remains of the star have collapsed into a body with a diameter of only tens of kilometres, with densities of the order $10^{14} \text{ g cm}^{-3}$ and possibly with magnetic fields of 10^{12} gauss.

It is this state of matter, hitherto merely a speculation of theorists, which is now observable, and the discovery must surely be acclaimed as entirely appropriate to the distinction now accorded to Hewish. **BERNARD LOVELL**



As if to show that astronomical research is truly an integral part of Western civilisation, the largest optical telescope in the Southern Hemisphere was officially opened on October 16 to the strains of a string quartet playing Purcell, Dvorak and Mozart. Prince Charles came from England to say the words which set the massive 326-tonne telescope slewing and the 526-tonne dome rotating in a bravura display of engineering gymnastics which left the 400 official guests (comfortably seated on the floor of the telescope) applauding in amazement at the scale of it all.

The ceremony was nicely done. The engineers and scientists were at pains to show that they can turn on a show worthy of princes and premiers and the \$A16 million of public money which it cost to build. For the people of the modest country town of Coonabarabran (population some 3,000), 29 km away, there had never been such a day. The town's five taxis had a field day (\$A3 to the airport, \$A8 to the telescope), the flags were out, the schoolchildren and the farmers met a real Prince, and the security men, obvious in their plainclothes, were there in force, giving rise to endless gossip.

Charter and VIP flights brought

hundreds from Sydney (340 km to the south-east) and Canberra. There was a batch of 16 British scientists, too, led by Professor Sir Fred Hoyle, present Chairman of the Anglo-Australian Telescope (AAT) Board, and Professor Sam Edwards, Chairman of the Science

Royal inauguration for AAT

from Peter Pockley

Research Council. They will have to work hard to keep up an Anglo semi-image to the project—but there will be no trouble over control and access to the telescope which was established on a very formal intergovernmental level with 50% shares each. In the popular imagination the AAT will always be depicted in the midst of the gum tree landscape of the Warrumbungle ranges, spectacularly spread out beneath Siding Spring Mountain which is the 1,165-m-high platform for the big dome and its four smaller companions (three Australian National University reflectors and the United Kingdom's own Schmidt telescope). Despite the careful 50/50 protocol of the opening ceremony and the words of Prince Charles and Prime

Minister Whitlam, the AAT will certainly become an Australian national symbol.

The description "largest in the Southern Hemisphere" is relatively easy to come by with feats of technology, for the land mass and population south of the Equator are but tiny fractions of the world totals. Yet this telescope is genuinely in the big league, and the situation of this giant eye where it can look right into the centre of the Galaxy and the Magellanic Clouds, the nearest galaxies to us outside our own, will give it an edge on its competitors (and collaborators) in the north which is sure to produce a spate of important observations. In the competitive astronomical stakes, though, the American 3.9-m telescope at Cerro Tololo in Chile will be only a few months behind the AAT.

The first calibrating photographs have already been taken before the mirror gets its first aluminising by the end of this month. Professor Ben Gascoigne, the Commissioning Astronomer, has enthusiastically pronounced the optics to be first class, better even than the specifications. He has now been joined at the AAT by the first Director, Joe Wampler, from Lick Observatory.

One European required for Spacelab

from Angela Croome

At least one European scientist must be chosen in the next six months to accompany and tend the European experiments aboard the first Spacelab—the orbital workshop designed and built in Europe, which is to be raised into space and returned by the American Space Shuttle, the prime space effort in the United States for the 1980s.

This is a deliberate phase-shift of a size that is scarcely appreciated, so quiet has been the final transition from automated satellites to a reusable man-in-space project. Europe seems to be slipping into the manned space programme almost by the backdoor. Yet a series of papers of the recent Astronautics Congress in Amsterdam show how fast the options are closing and what a brisk pace is being set.

The subtle combination of reliability, systems compatibility, miniaturisation, ruggedness and strict programme planning and management that space operations demand are recognised as an industrial challenge compatible only with a major weapons development or a nuclear programme and a means of forcing the pace both of technology and of management. This indeed has been one of Europe's principal motives for getting into the space business in a substantial way. Added to these are the implications of man-in-space, in an international context specifically. The requirements of a manned spacecraft are at least an order of magnitude more severe than for automatic satellites. Pressurisation, life-support, toxicity and flammability restrictions must all be considered, inevitably increasing design complexity.

The first Spacelab will fly in the spring of 1980 and the final choice for this mission must be made in the spring of 1975. In fact the experiments and the man-in-space to run them are a composite package. If, for instance, a major University of Southampton experiment in atmospheric physics requiring constant supervision is selected, then a Southampton experimenter would be expected to accompany the instrument and operate it. This means, say the NASA space medicine experts who have overall responsibility for man-in-space survival and performance, that Spacelab scientists will have to be part-qualified as astronauts to complete their tasks under zero-g and other disagreeable and unfamiliar space conditions—in short they will have to be under 55 and obtain experience of piloting high performance jet aircraft. An interesting challenge for typical European departmental pro-

fessors, one can say. The intention is that the first Spacelab payload will be shared roughly equally between Europe and the United States, with NASA having the final say in what flies and what does not.

More than 200 European proposals for the first Spacelab payload have already been received. In July the European space agency, ESRO, issued 200 invitations to appropriate industrial and academic bodies in Europe to put up ideas. Responses were due by the end of August but they are still flowing in. The German company Messerschmitt-Bölkow-Blohm is carrying out a preliminary screening, which includes tossing out proposals that are hopelessly incompatible or take insufficient advantage of Spacelab's special opportunities for bulky and relatively power-hungry experiments, and it will make its recommendations to ESRO in December. ESRO and NASA will then get together to define the first mission precisely and the final choice of experiments will follow from this.

The breakdown of the initial European proposals is interesting. By far the most numerous are technological and industrial rather than strictly scientific. (One German company has put up 20 proposals alone relating to communications.) The largest category is for space processing; 80 ideas have come in so far (more than twice as many as in any other category). There has been great interest in last year's American Skylab experiments in which pure crystals of such elements as germanium used in electronics components grew rapidly to diameters as great as 30 cm—impossible on Earth where growth is inhibited by gravity. There is speculation that space-grown crystals could actually bring down the high price of electronic components such as transistors and panels for solar heating. The production of special quality welds and of exotic alloys is also a possibility that European technologists wish to explore. Chemical separation by electrophoresis, possibly only under weightless conditions, promises greater purity in drugs and vaccines. Even an experimental space factory (requiring as it does bulky equipment, considerable power and a man in charge) has been outside the range of Europeans previously.

Earth resources and earth surveying is the second largest category of applications. It includes a novel experiment using a laser and a telescope to measure atmospheric scattering of light by gas particles; it will weigh two tons and consume one kilowatt of power. Two-thirds of the Earth-monitoring proposals come from Britain. Skylab experience has shown that a man in charge is a great asset for this type of survey from space for he can react

fast—for instance, refocus his instruments for detailed observation—when a special feature crops up.

The life sciences stand to benefit particularly from a supervised orbiting laboratory and there are plenty of ideas. A biological experiment is typically small and weighs little but it is expensive in human time.

The first Spacelab flight will be atypical. It will not orbit for more than a week and half the available power will be used to check out the sub-systems and procedures. It will be primarily a technical flight to make sure everything works. Consequently two out of the four payload staff will be equipment technicians, presumably provided from the American side. But the timetable for the second Spacelab lags only three months behind the first and 40% of all shuttle flights are expected to carry a Spacelab. Europe is now inextricably involved in the major manned programme for the 1980s and is being paced on a sharp timetable by NASA. For the United States, the space programme has proved a forcing house in engineering and management. Manned missions raise the game more for they cannot be allowed to fail. □

Ariel-5

THE fifth in a succession of British scientific satellites which have been put up by NASA, in the absence of any suitable indigenous rocketry, was successfully launched this week from the Italian San Marco platform off the Kenyan coast. Called Ariel-5 (UK-5 before the launch) the satellite is only the second in the world to be given over entirely to X-ray astronomy, although individual X-ray experiments have been carried on other satellites.

Ariel-5 has six experiments on board, five British and one American, and information will be fed to and from the satellite by the Science Research Council's Appleton Laboratory at Slough. Four of the experiments, designed by University College, London (two), University of Leicester and Imperial College, London, will be pointed at specific X-ray sources whereas the other two (University of Leicester and Goddard Space Flight Center) will scan large areas of the sky and suggest where it might be particularly interesting for the pointing experiments to look. X-ray sources which turn on unexpectedly will be monitored in this way.

Mr J. F. Smith, Programme Manager for Ariel-5 in Britain, said this week that the satellite is functioning extremely well. All the systems are working, he said, and the only problems are minor ones which will be ironed out in a few days. One group already has interesting scientific results to hand. □

correspondence

Checks and balances

SIR,—You raise the issue (*Nature*, September 6) of the role of journals in situations such as the proposed moratorium on plasmid engineering experiments. You conclude for two reasons that you should not, as a policy, refuse to publish any report of experiments that might be carried out in defiance of the call for postponement—this call, or others like it. Your two reasons were scientific variants of classic arguments against censorship: what referee can judge papers for their ethical standing? Why drive publication under ground? Yet you acknowledge implicitly the considerable power of journals such as yours to influence the standards—scientific, but perhaps ethical as well—of researchers. Is there a way to use your power not through editorial censorship but instead as an important auxiliary to the checks and balances that exist or may need to be set up in the scientific community? For a model, consider the statement you will find in the instructions to contributors to *Proceedings of the National Academy of Sciences*: "Manuscripts reporting experiments with humans should be accompanied by a copy of the document authorising the research by the responsible institutional committee."

American research hospitals and medical laboratories, as is generally known, have peer committees that must pass every plan for an experiment using human subjects, while there is still time—that is, before the experiment is begun. But one important aspect of the open letter by the American group was that the danger was imminent; as Paul Berg, a leader of the group, has said several times, many of the experiments at issue were already planned to be completed before the international meeting in February, and their dangers if any, reside not in some future consequence or application of published results but in the experiments themselves. Control must occur before the work, not merely before publication—not *imprimatur* but *lab-oretur*. The nuclear fission or "Hahn should not have published" analogy is misplaced. But many American and British biological laboratories, whose own people would be the first victims of an experiment gone wrong, are setting up biohazard or biological safety committees—scientific peer groups who will review research plans. Such review

is decentralised, appropriately motivated, and can be done early enough; it is done not just by peers every couple of years (as with peer review of grant applications in the American setting) but from week to week by colleagues who ought to be familiar with men, their work, and their equipment. Such institutional structures seem to me to be admirably adaptable to part at least of the present problem. As local safety committees begin to function, to reinforce them with an editorial requirement that reports submitted for publication be accompanied by evidence that the local review was done—like the rule at *PNAS* for human subjects—that would be a natural step with no taint of censorship.

Yours faithfully,
HORACE JUDSON

*Manting House,
Meldreth House, Cambridgeshire*

Joke with whiskers

SIR,—Martin C. Raff (*Nature*, 251, 184) describes nude mice which have spread through the immunological world at a remarkable pace. Should they be called squeakers?

Yours faithfully,
M. A. PERKIN

Rochdale, UK

For the birds

SIR,—Dr W. R. P. Bourne in his article "Egg Collector Collected" (*Nature*, 249, 793, 1974) has done well to draw the scientific world's attention to the egg- and bird-collecting outrages regularly perpetrated by ornithologists, some of high repute, in North America. I can vouch for his reference to a "conspiracy of silence"; leading publications in the field have declined to publish any mention of this disturbing subject. It is perhaps time that the rest of the world was made aware of the North American situation.

One might imagine that the National Audubon Society (NAS) would take a stand against this. After all, in the early days, were not two Audubon wardens murdered in defending egret nests in Florida? Yet when in 1969 two biologists shot 1,000 egrets in that state in the space of four weeks¹ this action was defended in a letter to me from the NAS by saying that it was all right because it was done by the "proper authorities".

What has been overlooked, however, is that experts in population dynamics (if experts they are) are not the only people with an interest in birds. To a great majority of people there are ethical and aesthetic considerations involved in taking the lives of so many beautiful and harmless creatures. This is perhaps an unscientific attitude; but birds do not belong to scientists and many people see no reason why, as in North America, ornithologists have some prescriptive right to kill them.

One might imagine that no ornithologist would raise any serious objection to a code that asked a collector to state, when applying for a permit, the nature of the scientific research he was engaged upon; what new information and understanding about avian biology he expected to gain from a study of his specimens; what species he required and how many; whether such information could be gained from a study of pre-existing specimens; and whether his collecting was likely to harm any population. The permit-issuing agency might then assess the scientific merit of the proposal and then issue a permit for a specified number of a named species for an approved purpose. Yet this suggestion has been repeatedly rejected by ornithologists in North America. Applicants are not obliged to state which species they wish to collect, open permits are issued without specifying which species may be shot or how many, and their holders may shoot any birds regardless of whether they are engaged in research on them or not.

Yours faithfully,
J. B. TATUM

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¹ Fogarty, M. J., and Hetrick, W. M., *Auk*, 90, 268 (1973).

Blameworthy

SIR,—In my article last week ("For those in peril on the factory floor") the Royal Society was unfortunately omitted from the list of organisations taking no interest in asbestosis and similar consequences of technology. In fact the Royal Society should have headed the list as the body with the greatest influence but the least inclination to do anything about it.

PETER J. SMITH

news and views

In pursuit of the transforming genes of adenoviruses

ANALYSING the functional activity of any large pieces of DNA seemed a prohibitive task until the advent of bacterial restriction enzymes. They have, without question, revolutionised the field of tumour virology, which was moving fast enough before their arrival, but which now produces important new facts at the drop of an enzyme. This is because, although the overall function of some animal tumour viruses, particularly of the papova and adenoviruses, was quite well understood, mapping of gene function by conventional genetics was proceeding very slowly, through surely. A method of selecting specific pieces of viral genomes to use as molecular probes has provided a way round this difficulty. With such probes it is immediately possible to say, for instance, whether the fact that transformed cells do not show complete virus expression is because the complete viral genome is not there. And if some of it is lost, then presumably that which remains contains those genes responsible for inducing or maintaining (or perhaps both) the transformed state. Questions such as these are at the core of current efforts to demystify the process of transformation, which, many people hope, itself bears some resemblance to tumour induction *in vivo*.

First to yield to mapping by restriction enzyme cleavage was the genome of SV40. Lagging somewhat behind, but now catching up rapidly, was analysis of the larger adenovirus genome. Three new papers highlight recent developments in the adenovirus field, using specific pieces of the genome produced by restriction enzymes of *Escherichia coli* (*EcoRI*) and of *Haemophilus parainfluenzae* (*HpaI*). Gallimore, Sharp and Sambrook (*J. molec. Biol.*, **89**, 49; 1974) have analysed the viral DNA in lines of rat cells transformed by adenovirus 2 (Ad2). Their work, together with that described in a previous paper (Sharp, Pettersson and Sambrook, *J. molec. Biol.*, **86**, 709; 1974), has led them to the conclusion that no Ad2-transformed rat cell contains the whole viral genome. This conclusion could not have been reached without specific pieces of adenovirus DNA to use in reassociation experiments, which are conducted as follows. Labelled probe DNA (whole or fragmented Ad2 DNA) is reannealed in the presence of unlabelled transformed cell DNA, and the increase in rate of reassociation produced by the viral sequences in the transformed cell DNA is measured. This increase is proportional to the number of viral genomes present. But using whole viral DNA as probe, there is no way of distinguishing between about one whole copy of the viral genome per cell and many copies of a small part of it—or any of the intermediate possibilities. Also, the frequency of different sections of the genome may vary, giving increases in reassociation rate which are averages. If restriction fragments are used as labelled probes instead, then the problem is vastly easier to disentangle. *EcoRI* enzyme produces six fragments of Ad2 RNA, one of which (fragment A) represents 58.5% of the genome, and is at the left-hand or GC-rich end. *HpaI* enzyme produces seven fragments, four

of which (fragments E,C,F and A) are in the *EcoRI* A fragment section of the genome. This is useful since a combination of *EcoRI* and *HpaI* treatment of adenovirus DNA allows dissection of *EcoRI* A fragment function. With all these thirteen fragments as labelled probes, Gallimore *et al.* measured their reassociation rates in the presence of DNA from nine different lines of transformed rat cells. Six lines show essentially the same situation: they contain sequences complementary to about 14% of the viral genome, all of which are at the left end and represent all or part of *EcoRI* fragment A, and *HpaI* fragments E and C. The number of copies of these sequences apparently varies between lines though, as the authors point out, this may be due to variations in the ploidy of the cells, which would make the figure used for DNA content per diploid cell inaccurate. Three other lines of transformed cells examined at the same time contain larger proportions of the adenovirus genome. No line contains a complete genome, and no line lacks the sequences at the left-hand end.

These findings are significant for a number of reasons. First, for lack of evidence to the contrary, people have tended to assume until now that transformed cells contain at least one viral genome. The only suggestion that this might not be so has come from the fact that there are transformed cell lines from which virus cannot be rescued by any of the available techniques. All the lines used by Gallimore *et al.* fall into this category. It is interesting that rat cells are also semi-permissive for adenovirus. Gallimore *et al.* argue that those cells which survive and become transformed are those which, for one reason or other, contain defective viral genomes. Possible reasons are either that the original infectious particle was defective, or else that part of the genome was lost after infection.

If the presence of viral genes is necessary for the maintenance of the transformed state, then the work of Gallimore *et al.* means that only 14% of the adenovirus genome is involved, since several of their cell lines contain only this much of it. Another way of approaching this problem would be to use specific fragments of the genome to induce transformation. It may be that the same left hand 14% of adenovirus DNA which is present in transformed rat cells contains all the information necessary to induce transformation in the first place. A year ago, Graham and van der Eb (*Virology*, **52**, 456; 1973) described a technique for assaying infectivity of Ad5 DNA and showed that it could transform rat cells (*Virology*, **54**, 536; 1973). In this issue of *Nature* (page 687) they report some results of their efforts to localise the transforming genes by using sheared adenovirus DNA as the transforming agent. The technique involves forming a co-precipitate of the DNA and calcium phosphate, which settles on to the cells, and from which DNA is taken up. Graham *et al.* demonstrate that whereas infectivity of adenovirus DNA is very sensitive to shearing, transformation can be induced by pieces only 1.0×10^6 daltons in size—about 5% of the whole genome ($20\text{--}25 \times 10^6$ daltons). Separation of the two halves of the genome (which have an asymmetrical distribution of GC) reveals that it is the left or GC-rich half which has most of the transforming activity. In attempting to localise this further, Graham *et al.* have followed the effect of progressive enzyme digestion from the two ends of the genome inwards. They conclude that because transforming activity is not affected

by digestion of the terminal 1% of the left end, but then decreases rapidly on further digestion, a segment extending from 1% in to about 6% of the genome comprises the transforming or 'T' segment. The results do not, however, really justify this degree of precision in defining the T segment. Its function could, for instance, be affected by destruction of sequences adjacent to it, and its actual position may not be so close to the left end as it seems. A much more convincing approach would be to use restriction fragments of adenovirus RNA as transforming agents. For instance, the *HpaI* E fragment makes up 4.1% of the left end of the genome and it, together with some of the adjacent fragment C, should contain the T segment. In the discussion at the end of the paper, such studies with restriction fragments are said to have been done; I hope their outcome will soon be published.

The work of Graham *et al.* reinforces the conclusions of Gallimore *et al.* that it is the left end, and probably the left 14%, of the adenovirus genome which is concerned with cell transformation. The experiments of Graham *et al.* provide a way of studying the induction of transformation by pieces of DNA which cannot code for more than two proteins, and may only code for one. The transcription of these sections of adenovirus DNA in transformed cells is now of even greater interest. Previous work has already shown that only a small percentage of the viral genome is transcribed in some adenovirus-transformed cells—a phenomenon also well documented for other DNA tumour viruses. The possibility that this is because most of the genome is actually missing as opposed to being repressed is an exciting one.

Another technique which has been essential to recent experiments on SV40 transcription is the separation of the two complementary strands of SV40 DNA (Westphal, *J. molec. Biol.*, **50**, 407; 1970). The technique depends on the asymmetrical transcription of twisted circular SV40 DNA by *E. coli* RNA polymerase. Using separated strands, it has been shown that sections of both strands of the DNA are transcribed during the lytic cycle (Khoury, Martin, Lee, Danna and Nathans, *J. molec. Biol.*, **78**, 377; 1973). It is not possible to separate the two strands of adenovirus DNA in the same way. Whole strands can be separated by differential binding of poly(U,G) to denatured Ad2 DNA followed by density gradient centrifugation. Tibbetts and Pettersson (*J. molec. Biol.*, **88**, 767; 1974) have now published a combination of this technique plus some kinetic trickery which produces labelled single strands of all the six *EcoRI* restriction



A sketch of Tasmanian Aboriginals done from memory by Annie Benbow in 1900. The last member of the race died in 1876; they had been moved from Tasmania to Flinders Island in the Bass Strait and the last 44 returned to a former convict probation station in Tasmania in 1847. Most of them died of influenza. (Records of the Queen Victoria Museum No. 49, 1974.)

fragments of adenovirus DNA. The technique is as follows. Large amounts of unlabelled whole complementary strands of Ad2 DNA are prepared as described above. A large excess of one whole complementary strand, for instance the heavy strand, is then mixed with labelled Ad2 DNA (whole or a specific restriction fragment), denatured and exhaustively renatured. What happens is that in the presence of a large excess of unlabelled heavy strands the amount of labelled complementary light strands becomes limiting, and at the end of the reaction up to 50% of the DNA remains single stranded. This residual single stranded DNA can be isolated by hydroxyapatite chromatography and corresponds to the heavy strands present in excess during the reaction. The important point is that it includes both labelled and unlabelled heavy strands, because the unlabelled heavy strands, being present in large excess, will preferentially renature, so effectively excluding their labelled counterparts from re-forming double-stranded molecules with their original partners, the labelled light strands. The technique of Tibbetts and Pettersson produces very pure single-stranded preparations, and much of their paper is concerned with establishing this and with analysing the kinetics involved. They then go on to use their preparations to show that in HeLa cells productively infected with Ad2, early cytoplasmic mRNA is complementary to 15% of the heavy strand and 30% of the light strand. Late mRNA is complementary to 22%

and 66% respectively. Of this late mRNA, most is transcribed from both strands of *EcoRI* fragments A,B and C, and from the light strand of fragments D,E and F. They discuss the implication that these areas of the genome may represent groups of genetic functions. Now the use of single-stranded restriction fragments should be extended to analyse transcription in adenovirus-transformed cells.

The three papers discussed in this review illustrate the application of three important techniques, namely, the use of restriction enzymes to produce specific fragments of viral genomes; the use of fragmented viral DNA to induce cell transformation; and the separation of complementary strands of viral DNA to study viral transcription. They also herald what could be the final campaign in the battle to analyse transformation from the viral point of view. Unfortunately, of course, transformation involves cells as well. Whether, when the viral DNA that is present in transformed cells is fully understood structurally and functionally, we shall also understand how transformation is induced and maintained remains to be seen. Tumour viruses should perhaps be considered as particularly useful triggers—because they can be analysed—for inducing cells to lose control of their division and growth. What we shall have when we understand their function is a model for studying essential cellular control mechanisms. The implications of this are immense.

ELIZABETH ROGERS

Fighting foot-and-mouth

from a Correspondent

THE Animal Virus Research Institute celebrated the completion of 50 years of research on foot-and-mouth disease at Pirbright by holding a symposium on September 26 and 27.

The changes in the world situation were reviewed by three speakers. Dr J. G. van Bakkum (State Veterinary Institute, Lelystad, Netherlands) presented the tables of prevalence of the disease in European countries in the last 50 years. During the major waves of disease—for example, in 1937/8 and in 1951/2—there were as many as 370,000 outbreaks in countries such as France and Germany. The development of the tongue tissue vaccine by Frenkel had made possible the introduction of prophylactic vaccination and campaigns were begun in Holland in 1952 and, within the next ten years, in France and Belgium. In each country the number of outbreaks fell dramatically, to under 10 yr^{-1} . There had been problems in relation to immunisation of pigs but these were being overcome and, in the recent outbreak in France, vaccine had played an important part in control. Europe still remained at risk through importation and through possible spread of the disease from Eastern Mediterranean countries but prospects for the control of ultimate eradication from Western European countries were good.

Dr J. H. Graves (Plum Island Animal Disease Research Laboratory, New York) dealt with current problems in the control of the disease in the Americas. In spite of the great efforts which have been made in extensive vaccination campaigns, results have not been as good as would have been expected on the basis of the European experience. A particularly difficult problem was the control of potency of vaccines used in the field and it was here that the work of the Pan American Foot-and-Mouth Disease Centre in Rio de Janeiro might be expected to influence the situation. This centre had already run many courses for laboratory workers in various countries in South America and, as a result, diagnostic methods had been improved and standardised. Similar action is now being taken on vaccine potency control. Dr Graves also referred to the new risks which were posed by the planned Pan-American Highway. Plans were being discussed for quarantine zones to prevent spread of the disease northwards.

Dr J. B. Brooksby (Pirbright) drew attention to the very uneven progress in control of the disease in countries in Asia and Africa. Extensive cam-

paigns have been carried out in the Soviet Union but the statistical information available is slight. In many other regions limited campaigns have begun. Perhaps the most encouraging of these were in relation to disease-free zones. A good example was in Kenya, where a start had been made with a limited number (fewer than 400,000) of animals and gradually extended as the campaign was shown to be a success. The zone in which the disease was not controlled included nearly two million animals. Though the total number of animals so far involved in Asia and Africa was trivial in comparison to the population, it was in such schemes that lay the greatest hope for ultimate control of the disease and the development of useful programmes for livestock improvement.

In another session, the biosynthesis of the virus and the structure of the virus particle in relation to its antigenic properties were reviewed by members of the biochemistry department of the institute. A paper by Dr K. M. Cowan (Plum Island Animal Disease Research Laboratory, New York) carried the studies of virus properties into the field of vaccines. The properties of two plaque variants isolated from cultures of virus of type Asia 1 in BHK cells were described. Selection occurred during routine passage, the large plaque variant being predominant in monolayer culture, where as the small plaque variant was favoured by growth in suspended cells. The small plaque variant was antigenically deficient in serological tests and also in its capacity to immunise cattle. This finding emphasised the practical importance of the method of culture of the virus for vaccine production.

The arithmetic of destruction

by David Davies

RARELY does the arithmetic of strategic nuclear weaponry surface for public discussion: words such as superiority, sufficiency and imbalance are reckoned to be all that a layman, or for that matter a politician, should require. All the more welcome then, is a recent pamphlet *Offensive Missiles* by Kosta Tsipis of the Stockholm International Peace Research Institute (Stockholm Paper 5). The author tries to form numerical estimates of destruction capabilities of the US and Soviet land-based missiles and concludes that the United States has been, is and will continue to remain well ahead.

As long as the accuracy of missiles was inadequate to guarantee that the opposition's missile silos would be wiped out by a pre-emptive strike, it was foolish for a potential aggressor to

target missile bases extensively, since the counterpunch aimed at cities could be so massive. Thus for many years targeting doctrine was centred on the destruction by both sides of cities. Gradually, however, the acquisition of missiles with higher accuracies capable of splitting in flight into several re-entry vehicles (MIRVs) each with its own target has enabled the possibility to be reconsidered of taking out all silos at each 'farm' and all farms simultaneously. But this operation depends crucially on accuracies and reliabilities being sufficient to ensure that few, if any, opposing missiles remain serviceable. That this is now on the cards is clear from, among other things, the declaration of the US Secretary of Defence, Mr Schlesinger, of the US government's intention to go for a counterforce capability.

Warheads can destroy missiles in their silos by a variety of methods: the overpressure from a blast is perhaps the most damaging effect and consequently silos are often hardened to withstand pressures up to several hundred pounds per square inch. Let H be the hardening pressure. In dealing with destruction probabilities the yield in megatons (y) and the circular error probability (CEP) in nautical miles appear in the ratios $y^3/(CEP)^2$ which call K , the lethality.

Then if n warheads are aimed at the same target, the probability of demolishing it with at least one warhead is

$$P = 1 - \exp \left\{ - \frac{Kn}{2H^3 \{fH\}^3} \right\}$$

The function $f(H)$ is slow varying and for H in the range 100–1,000 pound per square inch is roughly 0.15. Thus, for example, to have a probability of 90% of destroying a silo hardened to 1,000 p.s.i. a value of Kn of 71 is needed.

Tsipis makes the important point that countersilo capability of a strategic nuclear force is thus dependent on the product Kn summed over all missiles. This should allow us to calculate the relative abilities of the United States and Soviet Union to annihilate each other's missile forces. The figure shows how the two sides stand in 1974.

The weakest element in all this is knowledge of the accuracy of the re-entry vehicle as reflected quadratically in the CEP . The US values are fairly easily inferred from the open literature, but can the Soviet values—in all cases inferior to those of the US—be relied upon? Tsipis follows a policy of minimising the difference between Soviet and US accuracies: the Soviet value is the most optimistic, the US value the most pessimistic supportable by published figures. This may be sound policy, but it will raise a few eyebrows that Soviet missiles are so inferior to

American ones. Our only knowledge of Russian missile capability comes from US intelligence sources and this must surely be a suspect channel since there may be political reasons at any time to exaggerate or underplay opposition strength (only three years ago the same sources were erring by a factor of five in the relatively simple business of estimating the yield of Soviet nuclear tests). Since the value of K_n is so strongly dependent on CEP and since Tsipis is strangely unprepared either to

present values (see figure), so there is not yet a counterforce capability on either side.

Even if in the course of continuous improvement (including terminal guidance on to targets) either side gets the theoretical capability to demolish all the missile bases of the other side, there are reasons to believe that this could not be converted into an assured practical capability. Tsipis points out that there are at least two unknowns, one technical and one strategic.

some of its missiles, remains inviolate, as the location and destruction of submarines is still a highly chancy process. Thus either or both sides could then launch a salvo against cities from submarines, and bombers would also come into their own.

Do we know that either side would be caught flat-footed by a first strike? It seems unlikely that the attacked would fail to launch his missiles immediately the intention became clear—and these would be aimed at cities. Thus counterforce is a strategy that without extraordinary assurances would be extremely perilous, and (as Tsipis points out) only guarantees an ever-increasing expenditure on nuclear arms.

One point must be made in conclusion. SIPRI has been trusted over the past decade for its immensely impressive output of objective figures. It has recently, in my opinion, started to use the figures to pass value judgements on various governments. The trend is clear in the conclusion to the present booklet which contains gratuitous comments and guesses on the superpowers and their intentions. This diminishes the value of the report. I have already noted that the figures on accuracy are inevitably subjective: once views are expressed in a paper on the "unjustified and unnecessary" nature of US requests and on the doubtfulness that the Soviet Union will display "rational restraint" it is difficult to know whether these views have, even unconsciously, fed back into the subjective estimates.

Immunisation with steroids

from our
Steroid Biochemistry Correspondent

MUCH has been learnt about the biological role of hormones by surgical removal of the endocrine glands responsible for their secretion and administration of components of glandular secretions to determine the reversibility of the changes produced. But many glands secrete more than one hormone and it is desirable to selectively prevent or neutralise the secretion of a single hormone. Antibodies against specific secretions are now making this approach possible. For example, immunising an animal to its own steroid hormones reduces the concentration of circulating non-protein-bound steroid, the biologically active fraction, and prevents its interaction with tissue receptors.

Some studies with antibodies to the female sex hormones have already been reported in rats. This approach has been extended by Nieschlag *et al.* (*Acta endocr., Copenh.*, **76**, 556-569; 1974) to study the role of various

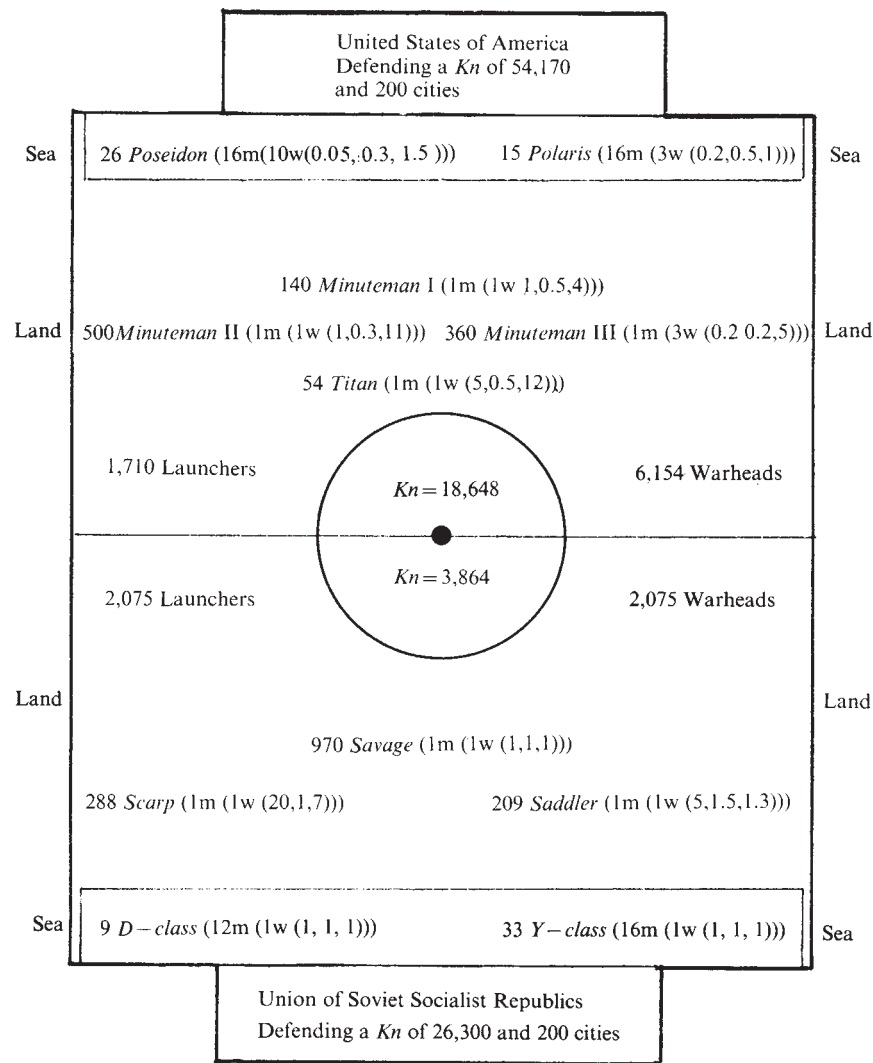


Fig. 1 How they line up: 54 *Titan* (1m (1w (5, 0.5, 12))) means there are 54 *Titan* sites, each with one missile, each missile carrying one warhead, each warhead being of 5 megatons, with an estimated CEP of 0.5 nautical miles and a K_n of 12. The total K_n to destroy all US silos is 54,170 (at 90% probability); the Soviet Union defends a value of 26,300.

substantiate his figures or to point out publicly the weakest link in the chain, it should be said that the figures consequent on CEP are one man's estimate.

If however they are to be trusted the inference is fairly clearly that whether by number of missiles (though not launchers) or by K_n , the United States is at present in a comfortably superior position, although Tsipis goes on to show that the value of K_n required to destroy all silos of either country with 90% probability is still larger than

The effect of one nuclear weapon detonating in the vicinity of a missile site is to make the delivery of a second a highly uncertain process in the period during which the fireball effects persist. On the other hand this makes the launch of missiles also a speculative process. An attacking nation must aim for a simultaneous attack on all silos in the firm with such precision that a second volley is unnecessary.

In all these calculations the submarine fleet, which may have saved

steroids in the control of testicular and adrenocortical function. As might be expected immunisation of rabbits with an antiserum to testosterone induced hypertrophy and hyperfunction of the testis as determined by histometry and measurement of serum testosterone levels. But the weight of the accessory reproductive glands of the testosterone-immunised rabbits was lower than that of control animals; the prostatic epithelium was atrophied and the diameter of the alveoli reduced showing neutralisation of the biological action of testosterone. Elevated concentrations of luteinising hormone and follicle-stimulating hormone were detected in blood and the changes produced were similar to those induced by administration of these gonadotrophins. This increased secretion of gonadotrophins from the pituitary seems to result from binding of the testosterone to the circulating antibody making it unavailable to the hypothalamic receptors.

In animals immunised against cortisol but not those receiving testosterone, androstenedione or oestradiol there was evidence for hyperfunction of the adrenal cortex. In rabbits immunised against oestradiol there was an increased secretion of luteinising hormone and Leydig cell hyperplasia of the testes similar to that observed after testosterone administration but in contrast there was no change in follicle-stimulating hormone levels, testicular weight or in accessory reproductive glands. The findings suggest therefore that oestradiol may have a differential effect on gonadotrophin secretion in the rabbit. The results of immunisation against other related steroids were more difficult to interpret because of some degree of cross reaction of the antibody with other hormones.

This way of investigating the role of various steroids in feedback control systems and their interaction seems very promising. It will no doubt be developed further in the future if more specific antibodies to a wide range of steroids can be developed.

Plants which survived glaciation

from Peter D. Moore

THE explanation of present day, disjunct distribution of plants and animals by reference to climatic changes during the Pleistocene is a process fraught with difficulties. European biogeographers have continually been at loggerheads with their geological colleagues concerning the likelihood of certain species surviving in isolated pockets or on solitary peaks during periods of major glacial

advance. In Norway a number of species show a bicentric distribution having stations on the northern and on the southern ends of the Scandinavian mountain chain, but being absent in the central area (for example, *Campanula uniflora*). Per-glacial survival in these two areas has been invoked as an explanation (Nordhagen in *North Atlantic Biota and their History*, edit. by A. Löve and D. Löve, 241, Macmillan, Toronto, 1963). Similar suggestions were put forward in the explanation of disjunct groups of arctic alpine plants in Britain, such as the Teesdale assemblage (see *Nature*, 249, 798; 1974). In both British and Norwegian situations, however, the problem may well be one of post-glacial disjunction owing to the extent of forest cover during the climatic optimum. The survival sites may be post-glacial rather than per-glacial refugia and may represent relict fragments of plant communities which were widespread at the close of the last glaciation.

In North America, a similar problem exists in the Gulf of St Lawrence area, where the disjunct distribution of some plants has been explained as the product of survival through the Wisconsin glaciation, but once again post-glacial forest spread could have been the agent of disjunction. In the west, parts of the Yukon have never been glaciated and are thus undisputed potential refugia for those species capable of survival under periglacial conditions (see Lindroth, *Endeavour*, 29, 129; 1970). Packer and Vitt (*Can. J. Bot.*, 52, 1393; 1974) have now proposed that the Mountain Park area of the Canadian Rocky Mountains in Alberta may have served as a glacial refugium.

Their argument is based mainly upon the collection of disjunct arctic-alpine and alpine plant species which are found together in this area, though they claim that geological evidence also supports the idea of an ice-free area existing throughout Wisconsin times. The ice-free regions would have included some foothill areas as well as protruding mountain peaks (nunataks). The disjunct species involved include some, such as *Eriophorum callitrix*, with a mainly arctic distribution, some, such as the bryophyte *Orthotrichum pylaisii*, which occur in both arctic and alpine situations, and some, such as *Telesonix jamesii*, which are essentially alpine and occur in isolated mountain sites mainly to the south of Mountain Park. A collection of disjunct species (the authors name sixteen), however, does not in itself prove per-glacial survival. There are two alternative explanations which must be examined first, namely long-distance immigration from a distant

centre of dispersal, and disjunction by forest invasion of territory once occupied by the relicts.

Long-distance dispersal is unlikely to account for all the species, since most have very poor dispersal mechanisms. If forest spread had caused the disjunction, then one would expect survival of at least some of the species at various sites between their now isolated stations, since at no time in the post-glacial period has the tree line been high enough to eliminate them from all geographically intermediate sites. They are, however, absent from these. The authors' hypothesis of *in situ* survival of the Wisconsin glaciation is thus the simplest available to explain the distribution.

One must still ask why the plants did not spread from this centre during the conducive climatic conditions of late-Wisconsin times, prior to forest invasion. It is difficult to conceive of severe genetic impoverishment occurring in populations isolated for a period (frequently interrupted) of at most 100,000 yr. One must hope that future palaeoecological studies of those species will supply more information on their glacial histories. Meanwhile, it is a matter for concern that this area is not included in any Canadian or provincial conservation programme and is currently threatened by mining.

Plumbing at the North Pole

from Peter J. Smith

ALTHOUGH the original soundings taken by Nansen (recorded in *Norwegian North Polar Expedition, 1893-96*, vol. 4, Jacob Dybwad, 1904) left no doubt that the Arctic Ocean is a deep water basin, the full complexity of the region has only become evident since modern surveys and traverses began in the later 1950s. Few would now disagree with Dawes (in *Implications of Continental Drift to the Earth Sciences*, Academic Press, 1973) when he says that "the basin is not a simple deep structure with uniform character but . . . is made up of individual basins separated by submarine ridges of contrasting morphology", although there would perhaps be less agreement on how the ridges were formed and hence on what they represent in terms of plate tectonics.

There are three ridges in the Arctic Ocean. The Lomonosov ridge is flat-topped and relatively narrow, rises to within 954 m of sea level, extends 2,000 km from the vicinity of northern Greenland and Ellesmere Island to the New Siberian Islands off the coast of Russia, and splits the total basin unequally into the Amerasian (larger)

and Eurasian (smaller) basins. The Amerasian basin, in turn, is divided unequally by the Alpha ridge, a broad fractured structure with rugged topography and complex morphology; and the Eurasian basin is cut roughly in half by the Nansen ridge, a seismically active offset extension of the mid-Atlantic ridge. Significantly, the three ridges are almost parallel to each other. Less significantly, the Lomonosov ridge is unique in that its edge lies beneath the north geographical pole.

All the evidence suggests that the Nansen ridge began spreading about 40 million years ago and continues to do so at a rate of about 1.0 cm a year; it has a clear geographical relationship with the mid-Atlantic ridge, is associated with a well defined pattern of earthquake epicentres, and gives rise to typical linear magnetic anomalies (albeit with smaller than usual amplitudes). The Alpha ridge, on the other hand, is evidently inactive in terms of both seismicity and spreading, although it is associated with linear magnetic anomalies of high amplitude. This last observation led Vogt and Ostenso (*J. Geophys. Res.*, **70**, 4925; 1970) to propose that the Alpha ridge, though now dormant, is the site of ancient seafloor spreading. According to this plausible interpretation, spreading would have begun about 200 million years ago (possibly simultaneously with the opening of the North Atlantic) and ended about 40 million years ago when the activity transferred to the Nansen ridge. Dawes tends to be more cautious about this, drawing attention to Vogt and Ostenso's own admission that the Alpha ridge may represent "something entirely different".

But if the Alpha and Nansen ridges are essentially oceanic features, the Lomonosov ridge is thought to be of continental origin, although it lies in

an anomalous geographical position. It is aseismic, has no linear magnetic anomalies associated with it and it is generally quiet magnetically—all of which suggests a scarcity of igneous rock. But this is negative evidence; and positive evidence in favour of a continental composition is more sparse. It is true that a seismic profile obtained by Ostenso (in *Implications of Continental Drift to the Earth Sciences*, Academic Press, 1973) indicates a smooth topography and a deep sedimentary structure; but again this relies for much of its effect on a comparison with known oceanic ridge structures. It is also true that positive, if indirect, evidence for the Lomonosov ridge's continental nature may be adduced from structures on the land adjacent to the Arctic Ocean, although, as Dawes points out with respect to the alignment of the ridge with geological features on Ellesmere Island, this support could prove to be ambiguous in the face of data suggesting that the ridge, although continental, originates elsewhere.

More positive evidence is thus urgently required—and has now been provided, within limits, by Lillestrand and Weber (*J. Geophys. Res.*, **79**, 3347; 1974). During the Second Canadian North Pole Expedition (1969) these workers measured the plumb line deflection in the vicinity of the Lomonosov ridge near the North Pole by comparing the paths of a drifting ice island station as measured astronomically and as determined by means of a Transit Satellite receiver. (The displacement between the two independently determined paths gives the plumb line deflection almost directly.) The mean deflection at 89° 40' N, 77° W was found to be 9 arcs towards 34° E, and the estimated deflection at the rotational north pole is 4–8 arcs in the direction 20–40° E.

This is apparently the first time that the plumb line deflection at the North Pole has been determined; and so the result is of interest in itself. But it is also important in connection with the nature of the Lomonosov ridge, for Lillestrand and Weber go on to show that the measured deflection is in good agreement with the value predicted from a model constructed on the assumption that the ridge is a sedimentary structure underlain by a lower density continental crustal block. Moreover, several gravity measurements made in the area are consistent with the free air gravity anomaly computed from the same model. Of course, all gravity-based interpretations are ambiguous in that any data set can be accounted for by a large number of different structures. Within this limitation, however, the new result from the North Pole cannot but strengthen

support for the view, first put forward by Heezen and Ewing (in *Geology of the Arctic*, University of Toronto Press, 1961) and later expanded by Wilson (*Nature*, **198**, 929; 1963), that the Lomonosov ridge represents a section of the Siberian continental margin split off when the Nansen ridge became active beneath the edge of Eurasia.

Diseases of skeletal muscle

from a Correspondent

MUCH of the work of the Third International Congress on Muscle Diseases (Newcastle upon Tyne, September 15 to 20) dealt with the interaction between nerve and muscle. Dr. L. Guth (National Institutes of Health, Bethesda) opened the meeting with a review of trophic interactions, dealing especially with cross innervation studies. Other workers discussed experiments utilising chronic stimulation of nerve or muscle both in animals and man to alter the histochemical, biochemical and physiological characteristics of muscle.

In a session on myasthenia gravis Dr E. Heilbronn (Stockholm) described the production of experimental myasthenia gravis in rabbits immunised with a purified preparation of acetylcholine receptor from the electric organ of *Torpedo*, and the effect of antibodies against this receptor. This will probably prove a fruitful model for the investigation of abnormalities of neuromuscular transmission. Various other immunological aspects of myasthenia gravis were considered, including the significance of HL-A antigens and various skeletal muscle and other antibodies. Dr G. H. Oosterhuis (Amsterdam) reported no correlation between thymic histology and the prognosis for myasthenia gravis.

A session was devoted to the neural theory of muscular dystrophy. Several workers, including Drs C. P. Panayiotopoulos (Athens National University), J. P. Ballantyne (University of Glasgow) and Professor W. G. Bradley (University of Newcastle upon Tyne) questioned the evidence upon which the theory was originally based. They considered both technical aspects and questions of logic; Professor A. J. McComas (McMaster University) defended the theory. Both Panayiotopoulos and Ballantyne reported studies using different techniques of motor unit counting in human muscle, finding no fall in the number of motor units in dystrophic patients compared with their own controls. The estimated number by their two different techniques was however not identical. Bradley reported that changes in the nerves of mice with muscular dystrophy were not found in

Democratic science

THE multiauthor paper has become a commonplace and the multinational project has come into its own. But authors usually achieve a consensus before publishing and present a united if sometimes cautious front. The latest multinational attempt to resolve some of the thorny problems of mycobacterial taxonomy (Meissner *et al.*, *J. gen. Microbiol.*, **83**, 207; 1974), however, has resulted in a published majority report with six out of the twenty-two authors presenting their own dissident statement on one of the proposals. Perhaps this approach could be encouraged; it would certainly make for more interesting reading in most scientific papers.

patients with Duchenne muscular dystrophy, though Dr Mukoyama (Nagoya) described some changes of the myelin sheaths in the spinal roots of such patients. Bradley's studies repeating the transplantation experiments originally taken to support the neural theory gave reason to suspect that the contraction of the host muscle might be recorded from the transplanted regenerate. Dr Neerunjun (Royal Postgraduate Medical School) however reported studies which supported the neural theory. Dr A. C. Peterson (University of British Columbia) described chimera experiments indicating that a skeletal muscle fibre might be morphologically normal though it had 96% dystrophic nuclei. From these experiments he concluded that the cause of murine muscular dystrophy lay outside the skeletal muscle. In discussion, the question was raised whether one or two per cent of normal nuclei might provide sufficient of a putative deficient substance to cure the disease. Dr M. Rathbone (McMaster University) showed that transplantation of foetal spinal cord from dystrophic chicks could produce biochemical enzyme changes indicative of dystrophy in otherwise normal chicks.

A session was devoted to papers bearing on the vascular theory of muscular dystrophy. Neither Drs O. B. Paulson and F. Jerusalem (Mayo Clinic, Rochester) nor Dr M. D. O'Brien (University of Newcastle upon Tyne) found decreases in muscle blood flow or capillary abnormalities in Duchenne muscular dystrophy. Professor K. Kunze commented that denervated human skeletal muscle shows similar or even lower P_{O_2} values when locally measured and compared with those in Duchenne and other muscular dystrophies. No defence of the vascular theory came in discussion from Dr W. King Engel (National Institutes of Health, Bethesda), though he restated his theory in a later review session dealing with aetiological theories.

In sessions on the clinical management of human muscular dystrophy, Dr I. M. Siegel (University of Chicago) reviewed the results of his enthusiastic and effective treatment of muscular dystrophy with surgery and surgical appliances. Dr R. H. T. Edwards (Royal Postgraduate Medical School) described a simple dynamometer for use in clinical studies of patients with muscle weakness. Dr J. B. Peter (University of California, Los Angeles) described a controlled trial of long-term high dose corticosteroid therapy in Duchenne muscular dystrophy. Initial results indicated a fall in the serum enzyme activities, but no certain clinical improvement to date.

Other sessions dealt with normal

muscle, with various forms of myopathy, malignant hyperpyrexia, and myositis, as well as the various animal models of muscular dystrophy. Biochemical studies of membrane abnormalities in myotonic conditions proved stimulating. Drs A. D. Roses and S. H. Appel (Duke University) reported various membrane abnormalities, including that of membrane protein phosphorylation in dystrophia myotonica. Dr R. M. Andiman (University of California, Los Angeles) found an increase of desmosterol in the membranes of animals rendered myotonic by various drugs, but no increase in human myotonic membranes.

Peripheral nerve studies in health and disease were fully considered in the congress. A session and a workshop were devoted to axoplasmic flow. Dr

S. Ochs (University of Indianapolis) reviewed the evidence for fast and slow rates of axoplasmic flow and Dr G. W. Gross (Munich) described his experiments on the garfish olfactory nerve, which led him to propound a theoretical model of the many features making up axoplasmic flow. Dr B. G. Livett (Monash University) (for Professor L. Austin) described experiments showing marked abnormalities of axoplasmic flow of proteins and lipids in the nerves of dystrophic mice. In a fruitful workshop on motor unit function, Professor E. Henneman (Harvard) described evidence that motor units have a regular and repeatable order of activation. This gave rise to considerable discussion, not all speakers being convinced by the evidence.

Mad March hares

from our *Animal Ecology Correspondent*

THE springtime behaviour of hares, normally peaceful and retiring creatures, is proverbial. During February, March and April they gang together, becoming bellicose, vocal and extrovert. The 'madness' lasts only a few weeks, beginning at the start of the breeding season but ending long before the breeding season is finished.

In a thorough study on the breeding biology of the European hare, G. A. Lincoln has brought to light some intriguing questions (*J. Zool., Lond.*, **174**, 1; 1974). The period of sexual quiescence in does lasts from September to January, and in bucks viable sperm occurs in the epididymides from mid-December to early September. The number of sperms present reaches a high level from February to June with the maximum testosterone content in April. Pregnancies were recorded every month from January to August with the maximum number of females pregnant and the highest mean number of fetuses per pregnancy occurring in April, May and June.

Lincoln found spermatozoa in cervical smears taken from does during November and December, well before the first ovulation of the season. (In this mammal ovulation is initiated by coitus: Stieve, *Zool. Anz.*, **148**, 101; 1952.) The hormonal complexion of the hare does not allow ovulation to occur until early January, so matings earlier than this fail to result in pregnancy. Perhaps such matings have a social function providing necessary courtship and mating experience for young animals.

'March madness' is thought by

Lincoln to be a rut having the function of stimulating the resumption of breeding activity among the does. Wynne-Edwards, however, believes it to be a form of self advertisement (*Animal Dispersion in Relation to Social Behaviour*, Oliver and Boyd, Edinburgh, 1962). The complex chasing and boxing of the 'madness' is a social means of establishing a breeding hierarchy amongst the males, possibly identifying socially inexperienced individuals from the rest. He argues that this must happen right at the start of the breeding season so that the year's "quota" of reproduction can be assessed. Both interpretations can be correct and verification must await further study. Certainly the behaviour has little immediate physiological effect as only 16% of March madness conceptions result in hares the following spring compared with between 39% and 45% from later conceptions.

The suggestion that a mechanism exists whereby young hares gain sexual experience before actually breeding has a parallel in at least one other species of mammal. Young male baboons mate with females at the very start of their oestrus cycles when the females' receptivity and chance of pregnancy is low. Towards the end of their cycles, females mate with older and more experienced males when their receptivity is high. Most pregnancies result from these late matings (Washburn and DeVore, *Scient. Am.*, June 1961). Perhaps even hares have a society with complex rules of conduct which offers to instruct young initiates in its mysteries.

articles

Chemical uniformity of airborne particulate material, and a maritime effect

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Airborne particulate material has a uniform elemental composition, in which the industrial elements are enriched relative to soil. In the surface of the North Sea the trace element content seems to be massively enhanced relative to bulk seawater.

DURING 1972–3 measurements of the trace element content of airborne dust, rainwater and the dry deposit, sampled at various places in Britain, following exploratory work at a single location^{1,2}, has yielded much basic information on the annual deposition inventory, the “washout factor”, and the “dry deposition velocity” which will be described in detail elsewhere^{3,4}. Meanwhile it is opportune to report observations that, first, demonstrate a remarkable uniformity in the elemental composition of the general aerosol and, second, suggest a considerable enhancement of the trace element content of the ocean surface.

The methods of sampling and analysis have been published previously² and will be restated only briefly. Samples of airborne dust have been collected by drawing air through a filter paper (Whatman 40) held in a polypropylene duct, which is capable in still air of collecting particles (specific gravity 1) of diameter up to about 150 μm . Rainwater is sampled by polythene funnel and bottle. Because the funnel is exposed continuously, both wet and dry deposition are collected. The dry deposition is collected separately on a horizontal sheet of filter paper (Whatman 541) sheltered from direct rain. Samplers are mounted at 1.5 m above ground, are run continuously and are changed each month. The location of the sampling stations is shown in Fig. 1.

Samples are analysed mainly by neutron activation followed by gamma-ray spectrometry but also by X-ray fluorescence, atomic absorption and chemical methods.

Chemical uniformity

The average concentrations in air during 1972 of about 30 trace and other elements at seven sites in Britain are tabulated in Table 1. The sites were chosen to represent rural and more industrial cases, although all are non-urban. The range of the values for an (non-maritime) element seem to span an order of magnitude or more. Now if the results from each site in Table 1 are referred to a single element, say scandium, then the ratios so obtained show much less variation from place to place (except for Na, Cl and Br). If in addition the concentration ratio of an element relative to scandium is normalised to the same ratio given by Bowen⁵ in average

soil then this “enrichment factor” shown in Fig. 2 demonstrates the reduction in variation from place to place. It also separates the elements into two classes, those that are “soil-derived” like scandium having factors about unity and those of “industrial” origin that are enriched relative to soil by a few to many times. There are two anomalies in Fig. 2—the large excesses of cobalt and nickel at Trebanos are probably due to nearby industrial sources. Obviously it is not difficult to seek excessive and anomalous values, for example, in a busy street in London during July 1972 the enrichment factor for lead was nearly 8,000 whereas the general rule from Fig. 2 is that the ratio of lead to scandium is sensibly constant over all the sites at a value somewhat more than 1,000 times that in nominally average soil. Enrichment factors may be derived from observations at more distant sites in the northern hemisphere. For example, elemental

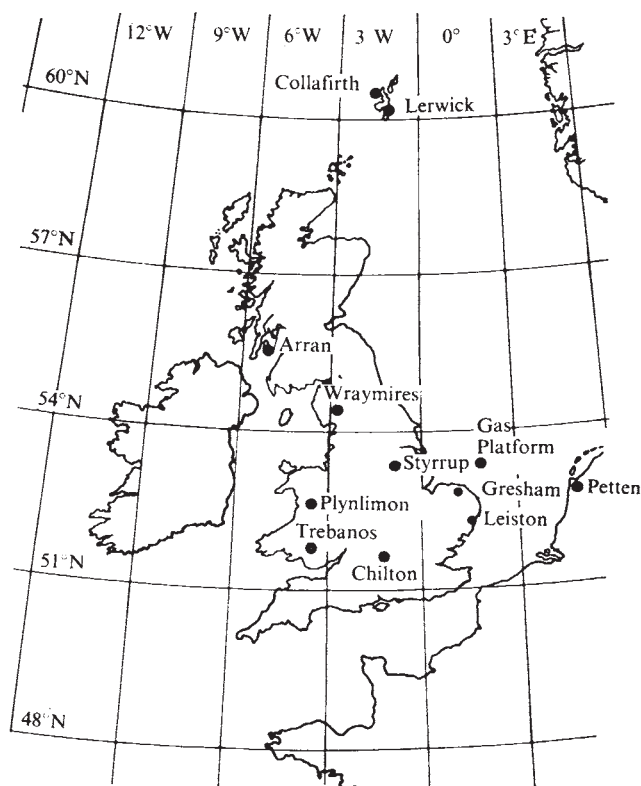


Fig 1 Location of sampling stations.

Table 1 Elemental concentrations in air (ng per kg air) January–December 1972

Element	Chilton Berks.	*Leiston Suffolk	Lerwick Shetland Is	Plynlimon Montgoms.	Styrrup Notts.	Trebanos Glamorgs.	Wraymires Lancs.	Average soil (ref. 5) (p.p.m.)
Na	760	990	1,900	800	900	1,000	540	6,300
Al	270	230	58	115	640	280	96	71,000
Cl	2,100	2,000	3,100	1,350	4,100	2,300	1,050	100
Ca	<610	<410	<550	<570	<1,100	<550	<260	13,700
Sc	0.065	0.058	0.014	0.029	0.18	0.102	0.029	7
V	14	11	2.3	4.2	21	14	5.0	100
Cr	4.4	7.5	1.1	2.0	17	9.6	1.5	100
Mn	20	20	3.1	9.0	47	18	7.8	850
Fe	310	340	67	140	940	430	140	38,000
Co	0.35	0.37	0.066	0.20	0.76	8.5	0.11	8
Ni	4.5	4.1	2.0	3.1	8.8	66	2.8	40
Cu	13	<9	<3	11	34	29	<6	20
Zn	155	160	39	76	340	190	56	50
As	5.1	4.9	1.9	2.0	24	7.0	2.7	6
Se	1.2	1.3	0.41	0.44	3.2	1.8	0.52	0.2
Br	39	31	13	11	130	40	16	5
Rb	<4	<3	<0.6	<1	<12	<8	<1	100
Cd	<27	<11	<7	<7	<34	<22	<16	0.06
In	<0.4	<0.3	<0.1	<0.2	<0.9	<1	<0.1	—
Sb	2.1	2.8	0.51	0.72	5.9	2.1	1.2	~6
I	<2	<1	<0.7	<2	<4	<4	<2	5
Cs	0.23	0.31	0.05	0.09	0.59	0.31	0.10	6
La	<0.3	<0.1	<0.3	<0.2	<0.6	<0.7	<0.7	30
Ce	0.36	0.32	0.07	0.14	0.70	0.50	0.15	50
W	<0.6	<0.5	<0.6	<0.3	<2	<0.9	<0.9	1
Au	<0.02	<0.01	<0.01	<0.02	<0.08	<0.04	<0.01	—
Hg	<0.3	<0.2	<0.04	<0.06	<0.4	<0.4	<0.1	0.03
Pb	130	110	29	43	330	190	56	10
Th	0.068	0.046	0.026	<0.02	0.12	<0.1	0.017	5

* February–December 1972

No adjustments have been made for filter efficiency².

analysis of air samples from the Osaka district of Japan⁶ can be summarised to give factors close to those reported in Fig. 2 for V, Co, Ni, Zn, As, Se, Sb and Pb. Another comparison using aerosol analyses by Rahn *et al.*⁷ from a remote site in northern Norway again illustrates the uniformity of enrichment factors for V, Cr, Zn, As, Se and Sb. In fact Rahn (private communication) by considering the distribution of enrichment factors worldwide, from remote to industrialised areas, has also demonstrated a general uniformity but with a sharp rise of enrichment factor at the highest elemental concentrations, that is, in the vicinity of a source.

The division of elements in Fig. 2 into soil-derived of low enrichment and industrial of higher enrichment con-

forms very well to the classification performed previously^{1,2} according to dry deposition velocity⁸, that seems to be correlated with particle size. The decrease of enrichment factor with increasing dry deposition velocity (rate of dry deposition/concentration in air) is shown in Fig. 3 for trace elements determined at Chilton. There is as yet no direct comparison at this site of particle size against deposition velocity. However preliminary measurements³ of the mass-median diameter at Chilton, using an Andersen sampler, confirm that deposition velocity increases with particle size and suggest that, at this site, a deposition velocity of 0.5 cm s⁻¹ corresponds to a mass median diameter of about 1 µm (specific gravity 1). This suggests that those trace elements that are enriched above the

Table 2 Elemental concentrations in air (ng per kg air) June 1972–May 1973

Element	Leiston Suffolk	Gresham Norfolk	Collafirth Shetland Is	Petten* N. Holland	Arran Bute	Lerwick Shetland Is	Gas platform N. Sea
Na	850	1,050	1,700	1,500	1,400	1,600	3,300
Al	210	240	44	155	130	44	155
Cl	2,000	2,500	2,700	2,600	2,400	2,600	5,700
Sc	0.054	0.065	0.016	0.042	0.038	0.010	0.067
V	11.0	10.0	1.75	9.0	8.8	1.85	9.7
Cr	5.4	3.4	1.05	3.1	2.8	0.67	3.7
Mn	19.5	21	3.2	14.5	9.6	2.5	17.5
Fe	310	320	70	290	220	49	270
Co	0.33	0.31	0.21	0.23	0.27	0.051	0.29
Ni	4.8	5.4	2.9	7.3	4.7	2.7	8.2
Cu	<11	<9	<4	<6	<5	<8	<17
Zn	120	105	25	75	105	24	125
As	5.2	5.2	1.20	3.7	1.85	1.30	4.2
Se	1.30	1.40	0.43	0.96	0.87	0.30	1.20
Br	74	69	19.0	60	33	20	73
Rb	3.1	3.1	<0.5	<3	<2	<0.4	3.3
Sb	2.9	2.4	0.49	2.5	1.30	0.38	2.4
Cs	0.25	0.21	0.037	0.155	0.115	0.026	0.17
Ce	0.30	0.29	0.077	0.175	0.195	0.054	0.20
Pb	130	130	31	96	71	23	120
Th	0.042	0.053	0.012	0.032	0.030	0.013	<0.04

* February–May 1973 only.

No adjustments have been made for filter efficiency².

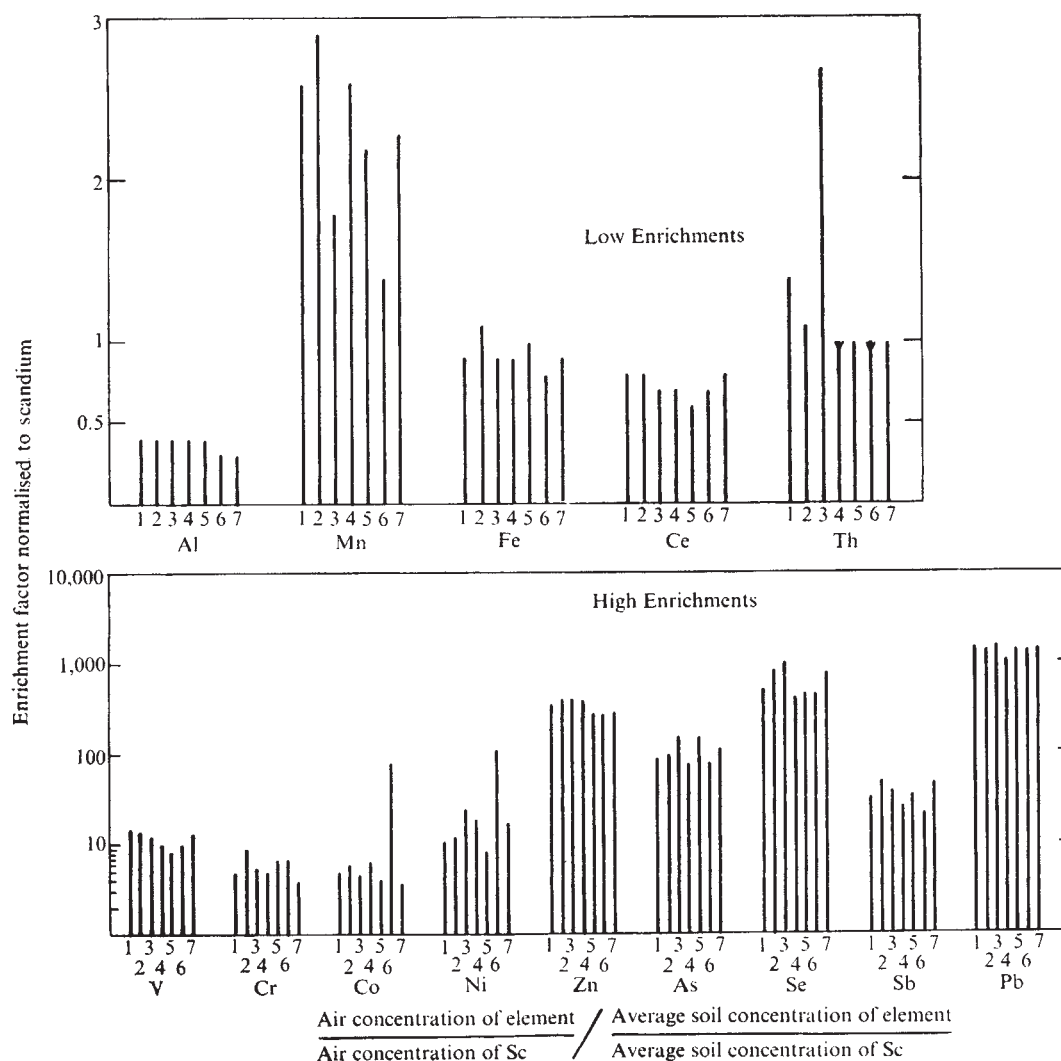


Fig. 2 Air particulate enrichment factors (1972). Sites 1: Chilton, Berks; 2: Leiston, Suffolk; 3: Lerwick, Shetland Is.; 4: Plynlimon, Montgomery; 5: Styrrup, Notts; 6: Trebanos, Glamorgan; 7: Wraymires, Lancs.

natural levels in soil appear as small particles, presumably formed by condensation after industrial combustion whereas natural particles formed by grinding and wind erosion are larger. But it is not impossible that the combustion of fossil fuels for example can produce both large and small particles.

If the results of the measurements presented above are representative not only of Britain but also of foreign sites in the same latitudes, then it is necessary to imagine and explain a general aerosol pervading this zone of latitude having a varying concentration but essentially the same elemental composition (except for the maritime elements Na, Cl and Br and also for regions in the vicinity of pollution sources, for example Trebanos, Fig. 2). It is difficult to postulate a mixture of such sustained uniformity from separate natural and industrial sources that generate particles of differing characteristic sizes. Material, that may be raised from the Earth's surface either by wind causing the suspension of soil and dust or by industrial processes, will probably lose particles of size greater than about 10 μm to gravitation. It is possible that enough of the particulate material below this limit will diffuse vertically upwards and join the westerly air-streams in the middle and upper troposphere at altitudes up to about 15 km. These air streams have been invoked to explain the passage of radioactive debris from nuclear explosions^{9,10}. Scavenging by rain will occur at and below cloud level (up to 3 km) and there will be continuous exchange by vertical diffusion throughout the troposphere. There is supporting evidence in the long range transport

of soils^{11,12}. Delany *et al.*¹³ measured vertical profiles up to an altitude of 9 km over continents and oceans, and found that over both, the component of the aerosol derived from the continents was dominant above about 2 km. It is reasonable to relate the aerosol behaviour described above to the "tropospheric background aerosol" proposed by Junge¹⁴.

There is thus a plausible explanation of the transfer of particulates from the surface and subsequent transport within the atmosphere, but not of the initial formation and subsequent maintenance of the mixture of elements. It is suggested, but cannot yet be proved, that the natural and artificial aerosols are produced by the separate processes, are mixed in the atmosphere, are circulated zonally and eventually reappear at the Earth's surface. Alternatively the aerosol mixture, or the part of it that is available for transport on a zonal or global scale, might be produced by a common process, not yet identified. After deposition the dust is available for re-suspension, more so from the paved surfaces of urban areas which are thereby exposed to higher concentrations of airborne dust than are rural areas.

Provided that the observed effect of chemical uniformity is real and is not caused by some undetected artefact of the sampling equipment, then two points of significance emerge. First, a scientific explanation of the formation of the aerosol mixture is required and could be pursued by a microscopic examination of the physical and chemical characteristics of the particles. Second, in relation to the monitoring of dust-laden environments, it should

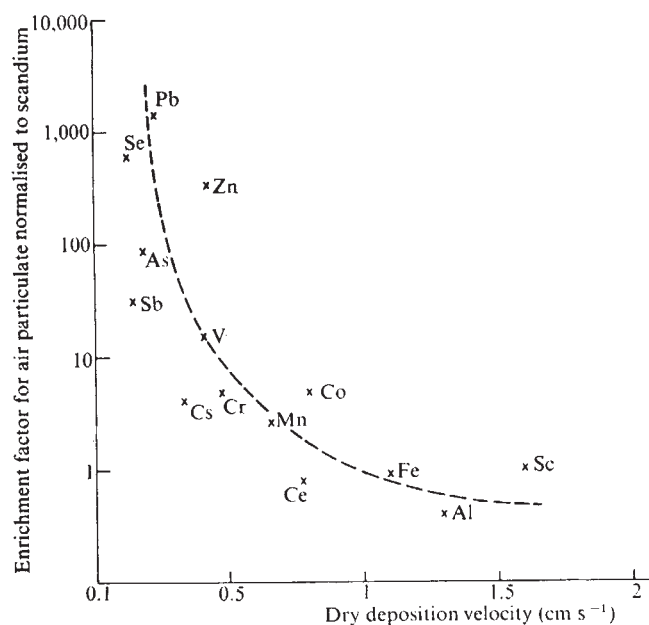


Fig. 3 Enrichment factor and dry deposition velocity at Chilton (Berks) 1972.

be possible by comparison with the "normal" elemental mixture to identify and define the presence of elemental "pollution".

Maritime effect

The observations of the effect of the ocean upon the atmospheric aerosol are less direct and comprehensive than in the case given above. The observations were made as part of a preliminary attempt to estimate the input of trace elements from the atmosphere into the North Sea⁴.

The average concentrations of trace elements in air and rain for a 12-month period during 1972–3 are shown in Tables 2 and 3. The sampling stations were sited at or near the east coast of Britain except for Arran in the west of Scotland, a platform at 25 m above the North Sea

about 55 km from the Norfolk coast and Petten in north Holland. The only noticeable trends in the air concentration results of Table 2 are that the values at Lerwick and Collafirth, being clean and rural, are generally lower than elsewhere except for the maritime elements Na and Cl. On the other hand the rain concentrations in Table 3 are higher by up to an order of magnitude at the platform in the North Sea than at the other sites, not only in the maritime elements but also in the other elements considered above to have soil and industrial origins. Such an enhancement would complicate any attempt to calculate the deposition of trace elements by rain into the North Sea.

There is no ready explanation of this effect. The high values of elemental concentration in rain obtained at the platform are general: it is unlikely that local contamination would elevate the values for all elements. Only Zn appears in excessive amounts and is a possible contaminant from the platform itself. Generally rain collectors situated on or near the sea would receive an amount of sea-spray that is significant compared with the actual rain that is collected. As has been demonstrated by Freudenthal¹⁹ for Sr-90, it is unlikely that this spray will scavenge trace elements from the atmosphere so much more efficiently than rain.

The most plausible explanation is that the excess of trace elements in rain has arrived with the sea-spray, driven by wind from the ocean surface. The air samplers do not register this excess presumably because the size of the spray particles is beyond the upper limit of collection of the sampling duct. The limit calculated for the downward-facing duct would be reduced in winds having large horizontal velocities.

The enhancement of the rain content by this maritime effect may be demonstrated by considering the concentration of an element in rain relative to that in air from Tables 2 and 3. This ratio ($\mu\text{g l}^{-1}$ of rain: $\mu\text{g kg}^{-1}$ of air), which takes account of the differences from place to place in the concentration in air, is equivalent to the washout factor used previously^{1,2,8}. The relation between this rain:air ratio and the seawater content of rain from site to site is demonstrated in Fig. 4 for several elements. With increasing seawater content, as indicated by the concentration of Na, the value of the ratio increases, typically by five times in the samples taken on the platform. This

Table 3 Elemental concentrations in rain ($\mu\text{g l}^{-1}$) June 1972–May 1973

Element	Leiston Suffolk	Gresham Norfolk	Collafirth Shetland Is	Petten* N. Holland	Arran Bute	Lerwick Shetland Is	Gas platform N. Sea	Seawater General (ref. 16)	North Sea (ref. 17)
Na	1,700	5,900	22,000	25,000	42,000	56,000	100,000	1.06×10^7	
Al	360	630	< 230	650	380	770	2,400	1.0–10	
Cl	3,000	12,000	46,000	45,000	93,000	105,000	210,000	1.9×10^7	
Sc	0.076	0.092	0.021	0.110	0.097	0.092	0.49	0.04	
V	4.4	9.0	< 8	8.9	< 7	< 6	< 40	2.0	
Cr	4.8	5.0	1.85	2.6	3.1	3.1	20	0.13–0.25	
Mn	12.0	21	< 5	23	< 17	< 24	< 60		0.24–0.54
Fe	370	440	135	570	540	420	3,100		0.06–1.5
Co	0.55	0.63	0.190	0.68	0.41	0.32	3.2	0.2–0.7	
Ni	6.4	6.8	3.8	7.2	6.0	5.4	23		0.16–0.51
Cu	28	27	23	41	27	23	82		0.24–1.70
Zn	260	210	26	150	130	110	2,000		1.5–6.9
As	1.25	4.7	< 8	12.0	< 6	< 17	45	3.0	
Se	0.48	0.69	0.40	0.46	0.39	0.91	1.75	4.0–6.0	
Br	33	130	360	390	460	1,300	2,300	6.5×10^4	
Rb	< 8	< 8	< 1.8	< 7	< 4	< 6	< 60	120	
Sb	1.20	1.10	0.30	2.0	0.46	0.67	5.9	0.5	
Cs	0.190	0.170	< 0.03	0.185	0.080	0.092	1.70	0.5	
Ce	0.53	0.53	< 0.1	0.55	0.43	< 0.5	< 4	0.4	
Pb	40	36	11.0	53	31	28	190		< 0.05–0.8
Th	< 0.08	< 0.10	< 0.07	< 0.11	0.21	0.135	< 0.7	0.5	
Rainfall mm yr^{-1}	417	476	1,542	516	1,129	1,201	260		

* February–May 1973 only.

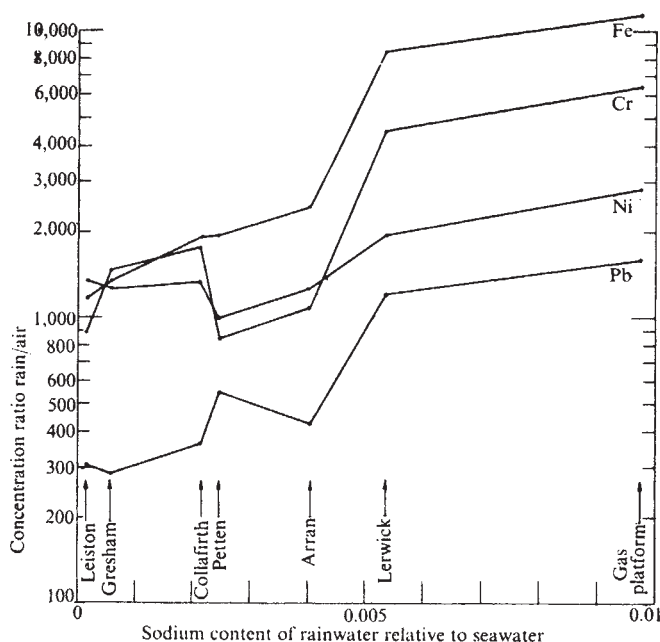


Fig. 4 Elemental concentration ratio, rain/air and seawater content of rain (1972–1973).

implies that most of the elemental material measured in the platform samples has been collected as spray from the surface of the sea. If the trace element content of these samples is compared with that in bulk seawater^{16,17} (Table 3) relative to Na, then it can be seen that the concentration of these trace elements, derived from the sea surface, is enhanced by several orders of magnitude compared with that in bulk seawater.

Duce *et al.*¹⁸ and Piotrowicz *et al.*¹⁹ have measured enrichments of up to 50 times in surface layers. The present observation is consistent with their results extrapolated to the surface microlayer and also with the results of Szekielda²⁰.

An excess of material on the ocean surface can arise in two ways, either by deposition from the atmosphere or by concentration of material from within the sea. The present work requires extension and elaboration to confirm these results, but if the enrichment of the sea surface

is a real effect then it would be logical to investigate the mechanism of enrichment and also the role of the ocean as a source of material for transport in the atmosphere, locally and on a much larger scale.

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Pitcairn Island—another Pacific hot spot?

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The volcanic Pitcairn Island, which has surface lavas with apparent ages between 0.45 and 0.93 Myr, may be the youngest member of another NW–SE trending volcanic lineament in the Pacific Ocean.

PITCAIRN Island, in the south-eastern Pacific Ocean (24°04'S, 130°06'W), is built entirely of volcanic rock¹. Unlike the majority of volcanic islands in the Pacific basin, which are

grouped in distinctive linear chains (see Fig. 1), Pitcairn is isolated from its nearest neighbours, Oeno, Ducie and Henderson (all coralline islands) by 160 km, and it is 600 km from its closest volcanic neighbour, Mangareva in the Gambier Islands. Easter Island lies approximately 2,100 km to the east, near the summit of the East Pacific Rise. The position and youth of Pitcairn Island beg explanation by one or another of the rapidly proliferating theories of nonorogenic volcanism.

Pitcairn Island is built up from the seafloor, a depth of at least 3,500 m (ref. 2). The age of the oceanic crust on which

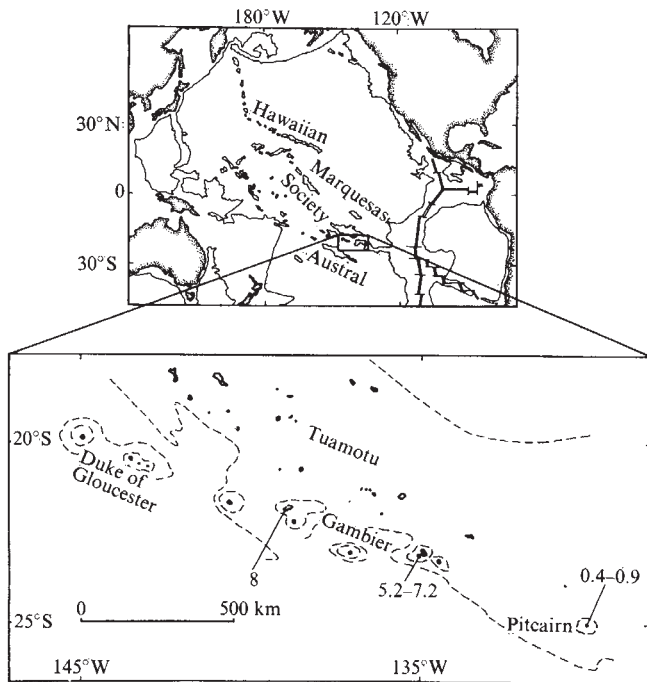


Fig. 1 Pitcairn Island is situated in the Pacific Basin, possibly the SE end of the Duke of Gloucester–Gambier Islands volcanic lineament which is parallel to other well known Pacific Island chains (Hawaiian, Marquesan, Society and Austral). The Duke of Gloucester Islands are all coral atolls at sealevel. Numbers indicate K–Ar ages $\times 10^6$ yr. Bathymetry in the Pacific Basin denoted by the 4,000 m contour; in the inset by the 4,000 m and 2,000 m contours (dotted lines).

the island is constructed is not known with any great precision but magnetic anomaly studies³ predict an age close to 30 Myr (anomalies 7–9). Basal sediments from Deep Sea Drilling Project (DSDP) site 75, 1,200 km NNW of Pitcairn (and roughly the same distance from the East Pacific Rise), have been assigned a lower Oligocene age (35–40 Myr)⁴.

The island is approximately elliptical, measuring 4 km by 2 km and is cliffed on all sides, rising to an altitude of 347 m

(Fig. 2). Volcanic rocks of two types—lavas and pyroclastics—predominate. Structurally, the island seems to be the remnant of a single shield volcano, its dissected caldera rim forming a prominent annular ridge which opens to the north. Four units have been discriminated: the Tedsides Volcanics—gently dipping flows exposed near the west coast; the Christians Cave Formation—an agglomeratic tuff generally overlaying the Tedsides Volcanics with erosional unconformity; the overlying Adamstown Volcanics—horizontal flows which fill the central basin; and the Pulawana Volcanics—a sequence of lava flows at the western extremity of the island, which may be coeval with the Adamstown Volcanics, as their lithology suggests. There are also some dykes, mainly within the Tedsides Volcanics.

Petrologically, the lavas range from alkali olivine basalts through hawaiites and mugearites to minor trachytes. The basalts contain phenocrysts of plagioclase feldspar, olivine and augite. The hawaiites and mugearites exhibit trachytic texture and contain fewer phenocrysts than the basalts. A common additional groundmass phase in these samples is biotite. The alkaline character of the trachytes is evident in phenocrysts of alkali feldspar, aegirine augite and fayalitic olivine. Almost all of the rocks contain interstitial alkali feldspar, the amount increasing in the sequence olivine basalt to trachyte. Chemical analyses of hawaiites, mugearites and trachytes from Pitcairn Island have been presented by Lacroix⁵ who finds them comparable to the petrological association in the Marquesas Islands^{6,7}. New analyses from this Pitcairn collection will be published elsewhere.

Geochronology

The samples selected for dating in this study are primarily hawaiites and mugearites. All four lithological units are represented in the age determinations.

Of the 17 selected samples, 12 were chosen for K–Ar age determination, on the basis of thin section examination. Only those specimens which were devoid of any alteration of the K-bearing phases were selected. Generally, they were holocrystalline and nonvesicular.

Measurements were taken following the techniques previously described by McDougall^{7–9}. Constants used in the calculations are: $\lambda_e = 0.585 \times 10^{-10} \text{ yr}^{-1}$; $\lambda_\beta = 4.72 \times 10^{-10} \text{ yr}^{-1}$; $^{40}\text{K}/\text{K} = 1.19 \times 10^{-2} \text{ atom } \%$.

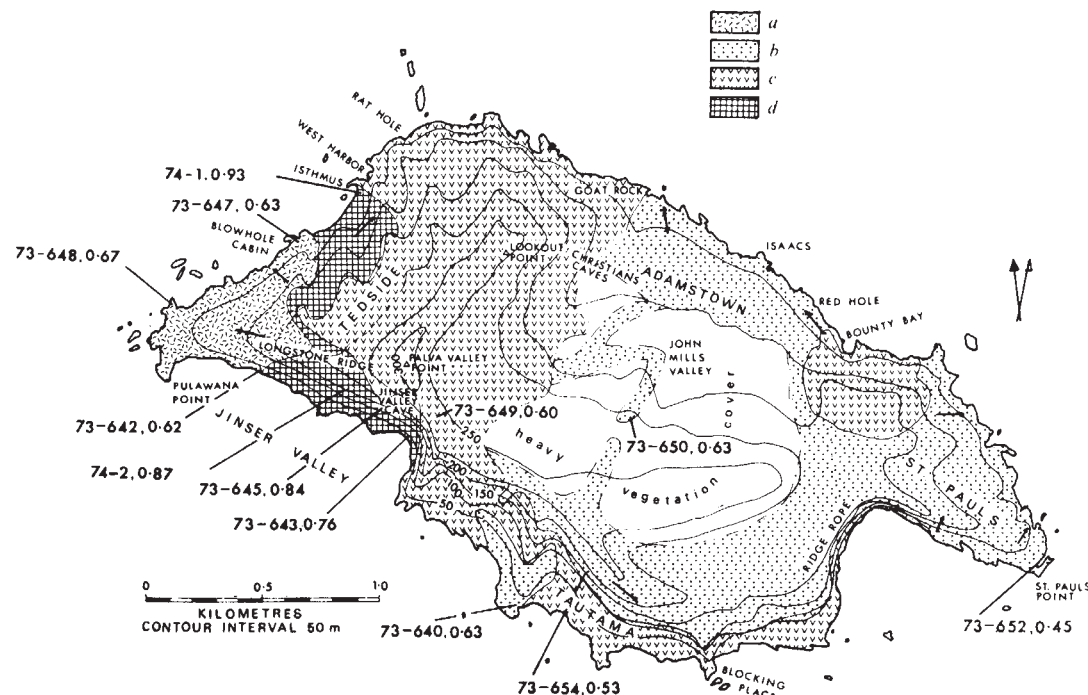


Fig. 2 Generalised geology of Pitcairn Island (after Carter¹). a, Pulawana Volcanics; b, Adamstown Volcanics; c, Christian Cave Formation; d, Tedsides Volcanics. Arrows indicate the generalised direction of lava flows. Observed geological contacts indicated by solid line; inferred contacts, dashed line. Numbers after commas, K–Ar ages ($\times 10^6$ yr) of samples indicated. Contours at 50 m intervals.

Table 1 K-Ar ages on whole rock samples from Pitcairn Island

Laboratory No.	Otago University No.	Rock Type	K (weight %)	rad. ⁴⁰ Ar 10 ⁻⁸ cm ³ NTP g ⁻¹	100 rad. ⁴⁰ Ar Total ⁴⁰ Ar	Calculated age $\pm$ 2 s.d. (Myr)	Approximate altitude (m)	Locality
Pulawana Volcanics								
73-647	18815	Hawaiite	1.694; 1.696	4.27	32.5	0.63 $\pm$ 0.01	25	Cabin Cave, NW coast
73-642	18740	Hawaiite	2.183; 2.169	5.42	28.8	0.62 $\pm$ 0.01	10	Jinser Valley, SW coast
				5.41	28.9	0.62 $\pm$ 0.02		
73-648	18823	Trachyte	3.826; 3.828	10.28	34.8	0.67 $\pm$ 0.01	10	Dyke, W coast
Adamstown Volcanics								
73-652	18878	Hawaiite	1.537; 1.535	2.80	20.8	0.46 $\pm$ 0.01	30	St Pauls Point, Eastern tip of island
				2.78	16.7	0.45 $\pm$ 0.02		
73-654	18888	Biotite mugearite	1.718; 1.736	3.75	9.0	0.54 $\pm$ 0.04	110	Artefact site, Tautama, adjacent to south coast
				3.61	18.0	0.52 $\pm$ 0.02		
73-650	18825	Olivine mugearite	2.315; 2.311	5.63	26.0	0.61 $\pm$ 0.02	200	Western side of John Mills Valley, centre of island
				5.96	36.7	0.64 $\pm$ 0.02		
73-640	18730A	Mugearite	2.024; 2.036	5.11	31.7	0.63 $\pm$ 0.01	20	Western end Tautama, southern coast; possibly Adamstown Volcanics
Christians Cave Formation								
73-649	18824	Hawaiite	1.274; 1.286	3.04	19.5	0.60 $\pm$ 0.02	270	Block in agglomerate, Palva Ridge
Tedside Volcanics								
74-1	18755	Basalt	1.429; 1.418	5.41	40.5	0.95 $\pm$ 0.02	10	Water Valley
				5.23	38.3	0.92 $\pm$ 0.02		
74-2	18731	Hawaiite	1.960; 1.964	7.03	16.2	0.90 $\pm$ 0.03	150	Jinser Valley
				6.65	51.3	0.85 $\pm$ 0.01		
73-645	18764	Hawaiite	1.491; 1.496	5.01	46.0	0.84 $\pm$ 0.01	130	Jinser Valley
				5.01	47.3	0.84 $\pm$ 0.01		
73-643	18753	Basalt	1.065; 1.067	3.23	42.1	0.76 $\pm$ 0.01	30	Jinser Valley
				3.28	34.5	0.77 $\pm$ 0.02		

Table 1 presents the analytical data for 20 K-Ar age determinations on the 12 whole rock samples. Generally, the duplicate analyses on 8 of the samples show agreement to within experimental error. The samples are grouped according to the formations defined by Carter¹. Localities and ages are shown in Fig. 2.

The calculated ages fall into at least two groups. Ages for four samples from the Tedside Volcanics, stratigraphically the oldest unit exposed on Pitcairn, form a group distinctly older than the rest, averaging 0.85 Myr and spanning the period 0.76–0.93 Myr. The oldest apparent age was obtained on sample 74-1 from the north-west coast (near sealevel), about 1 km from the other three Tedside collecting localities which were in Jinser Valley. The three Jinser Valley samples are from nearly horizontal lavas. Samples 74-2 and 73-645 are from approximately the same stratigraphical horizon and give nearly identical ages, averaging 0.86 Myr. The third sample (73-643), stratigraphically lower in the sequence, yields a significantly younger age of 0.76 $\pm$ 0.01 Myr. (Because minor interstitial glass occurs in this sample it is possible that some radiogenic argon has been lost from it, resulting in a younger apparent age.) These data suggest that much of the exposed Tedside Volcanics were erupted over a short interval of time between about 0.85 Myr and 0.93 Myr ago.

The samples from the Christians Cave Formation, and the Adamstown and Pulawana Volcanics all yield younger K-Ar ages—from 0.46–0.63 Myr—consistent with their stratigraphically younger age. A block from an agglomerate of the Christians Cave Formation yields an age of 0.60 Myr, indistinguishable from the apparent age of 0.62 Myr for sample 73-650 from the overlying Adamstown Volcanics. The Christians Cave formation can thus be interpreted as contemporaneous with an early phase of the Adamstown Volcanics. Sample 73-640 gives a similar age (0.63 $\pm$ 0.01 Myr) and was collected from the south coast. The radiometric age supports its inclusion in the Adamstown Volcanics as tentatively suggested by Carter¹. The two remaining samples from the Adamstown Volcanics have measured ages that are significantly younger (0.53 Myr and 0.45 Myr). Sample 73-654 is from a biotite mugearite flow, from which samples were taken by early Polynesians to manu-

facture artefacts. The Pulawana Volcanics, which overlie stratigraphically the Tedside Volcanics, near to the west coast, give concordant K-Ar ages of 0.62–0.63 Myr on two samples. A trachyte dyke (73-648) cutting the Pulawana Volcanics near the western tip of the island gives a slightly older age of 0.67 $\pm$ 0.01 Myr. These ages are indistinguishable from the older ages found for the Adamstown Volcanics, which supports the suggestion² that the two formations are in part equivalent.

We interpret these data as indicating that the Tedside volcanics, regarded as a relic of a dome building phase, were erupted between about 0.8 Myr and 0.9 Myr ago. Following a hiatus of about 0.2 Myr, during which time caldera subsidence occurred, the Christians Cave Formation, the Adamstown Volcanics (which filled the caldera), and the extracaldera flows of the Pulawana Volcanics, were erupted about 0.62 Myr ago, with volcanism continuing until at least about 0.45 Myr ago. The volcano was therefore active for a period of 0.5 Myr during the younger part of the Pleistocene.

The Adamstown Volcanics and Christians Cave Formation are normally magnetised¹, which is consistent with their geochronological placing in the Brunhes normal magnetic epoch. This study suggests that the Pulawana Volcanics also should have normal polarity. Field compass texts indicated that the Tedside Volcanics may be normally magnetised. This suggests that Tedside Volcanics were erupted during the short Jaramillo normal polarity event (about 0.90 Myr BP) during the Matuyama reversed polarity epoch.

Migration of volcanism

Potassium-argon age determinations indicate that the exposed lavas on Pitcairn Island were erupted between 0.45 Myr and 0.93 Myr ago, and the island is therefore a relatively young feature in the south-eastern Pacific Ocean. Its young age is incompatible with the estimated age of the surrounding sea floor and we conclude that Pitcairn Island could not have been formed at the East Pacific Ridge and then drifted north-west. Its relationship to other areas of recent volcanism within the Pacific Ocean basin—the islands at the south-eastern end of the conspicuously linear and almost parallel Pacific

island chains (the Hawaiian Islands, the Marquesas Islands, the Austral Islands and the Society Islands; Fig. 1)—is not immediately clear, because of the absence of a neighbouring chain of volcanic islands. The possibility that a nearby seamount chain exists, cannot be dismissed until more detailed bathymetry has been undertaken. Pitcairn Island lies, however, very near to the south-eastern extension of the Duke of Gloucester Islands–Gambier Islands lineament, which is also approximately parallel to the Pacific island chains. Potassium-argon age determinations on volcanic samples from the Gambier Islands¹⁰ suggest that the tholeiitic and alkali olivine basalt lavas which form the islands of Mangareva, Aukena and Makapu, cooled between 5.2 Myr and 7.2 Myr ago. Winterer¹¹ has reported an age of 8 Myr (D. Krummenacher, personal communication) for basalt from a drill hole at Mururoa Atoll at the north-western end of the Gambier Islands. Thus, there is the suggestion of a NW–SE migration of volcanism, which supports other geochronological studies of linear Pacific island chains^{12–15}.

If Pitcairn Island is a more recent manifestation of the source which produced volcanism in the Gambier Islands (and earlier, presumably, in the Duke of Gloucester Islands), then that source has migrated relative to the Pacific plate at a rate of approximately 11 cm yr⁻¹. This value falls within previously determined migration rates of Pacific island chains, which range from 15 cm yr⁻¹ in the Hawaiian Islands¹⁵, through

10 cm yr⁻¹ in the Marquesas Islands¹², to 9 cm yr⁻¹ in the Austral Islands^{13,14}.

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Methylation of 16S RNA during ribosome assembly *in vitro*

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Methyl deficient 16S RNA from kasugamycin resistant Escherichia coli has been used to study methylation of RNA during ribosome assembly in vitro. Methylation occurs at an intermediate stage and is inhibited at a late stage of assembly. 30S ribosomal proteins required for methylation, and inhibition, have been identified.

RI BOSOMAL RNA is synthesised *in vivo* as a large precursor which is reduced in length and methylated during ribosome assembly^{1–6}. Sequential addition of ribosomal proteins and conformational changes occur during maturation, resulting in the formation of mature ribosomes⁶. Nomura and coworkers have studied the *in vitro* assembly of ribosomes using fully methylated mature 16S RNA. Little is known, however, about the role of ribosomal proteins in the processing of precursor 16S RNA⁷. Recent experiments which demonstrated that 16S RNA from kasugamycin-resistant *E. coli* is undermethylated, provide an approach to an understanding of methylation and the sequence of events that occur during assembly of 30S ribosomes.

Mutations at the *ksgA* locus in *E. coli* result in kasugamycin resistance due to the lack of a methylase activity^{8–10}. Helser *et al.* demonstrated that resistance or

sensitivity to the antibiotic kasugamycin is determined by the nature of 16S RNA; 16S RNA from the resistant strain differs from the sensitive strain in that it lacks dimethylation of two adjacent adenines in a sequence of m²A m²ACCUG near the 3'–OH end of the molecule⁸. Helser *et al.* have also demonstrated a direct cause and effect relationship between adenine dimethylation and sensitivity to the drug by first methylating the resistant 16S RNA *in vitro* followed by reconstitution of 30S subunits with this RNA⁹.

Isolated 'kasugamycin-resistant' 16S RNA could not be methylated *in vitro* in these studies, but the ribonucleo-protein particles prepared by CsCl density gradient centrifugation (CsCl cores) were active substrates. This is consistent with the notion that methylation occurs during ribosome assembly. We report here our studies on methylation of 16S RNA from a kasugamycin-resistant strain during 30S ribosome assembly *in vitro*.

Purification and properties of methylase

The methylase was purified from *E. coli* A19 (RNase I⁻, General Biochemicals). Cells were disrupted in a French press in TMAI buffer (10mM Tris-HCl, pH 7.6, 30 mM NH₄Cl, 10 mM MgCl₂ and 6 mM 2-mercaptoethanol), the ribosomes separated by centrifugation and washed overnight in IF buffer (20 mM Tris-HCl, pH 7.8, 30 mM MgCl₂, 1 M NH₄Cl, 6 mM 2-mercaptoethanol). The salt-washed

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ribosomes were pelleted and the high speed supernatant was used for purification of the enzyme. The supernatant was dialysed first against TMAI buffer overnight and then against phosphate buffer (50 mM NaH_2PO_4 , pH 5.8, 1.5 mM 2-mercaptoethanol) containing 10% glycerol and adsorbed on to a phosphocellulose column equilibrated with the same

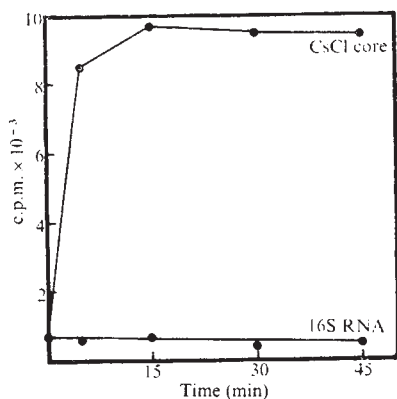


Fig. 1 Time course of methylation of CsCl core particles obtained from kasugamycin-resistant 30S ribosomal subunits. 0.25 A_{260} units of CsCl core particles (16.6 pmol) or 16S RNA (20 pmol) were incubated in 30 μl of reconstitution buffer (30 mM Tris-HCl, pH 7.4, 20 mM MgCl_2 , 6 mM 2-mercaptoethanol and 330 mM KCl) for 30 min at 40° C. These conditions are referred to as 'reconstitution conditions'¹². The tubes were then chilled on ice and 5 μl of partially purified methylase ($\sim 1 \mu\text{g}$) and 1 μl of ^3H -methyl-labelled S-adenosyl L-methionine specific activity 500 $\mu\text{Ci } \mu\text{mol}^{-1}$ were added. The reaction mixtures were diluted to 100 μl with methylase buffer (50 mM Tris-HCl, pH 7.8, 45 mM NH_4Cl , 4 mM $\text{Mg}(\text{OAc})_2$) and assayed for the indicated times at 37° C. The reaction was stopped by adding 1 ml of 10% trichloroacetic acid (TCA) and 0.2 ml of 0.2 mg ml^{-1} bovine serum albumin. The precipitates were collected, washed with 5% TCA and counted. Under these conditions, 10,000 c.p.m. correspond to 68 pmol of methyl groups incorporated. (About 5–10% background radioactivity was usually observed in RNase treated controls. These background counts were not subtracted from the data presented. The RNase sensitive maximum methylation corresponds to 3.3 methyl groups per core particle.)

buffer. The column was washed with phosphate buffer containing 0.25 M NaCl and the enzyme was eluted with a linear salt and pH gradient (0.25–0.65 M NaCl; pH 5.8–6.5). The methylating activity eluted as a major peak near 0.55 M NaCl with a small shoulder. The major peak fractions were pooled and dialysed against TMAI buffer (containing 10% glycerol) and used as a source of methylase. The conditions for assay of column fractions for methylase activity were as reported previously⁹. The methylase obtained by this procedure is 60–80-fold purified over crude extracts and remains stable when frozen at –70° C in TMAI buffer containing 10% glycerol. Further purification of the enzyme resulted in loss of activity, hence the phosphocellulose enzyme was used in all experiments. 30S ribosomes, CsCl core particles and 16S RNA were prepared from kasugamycin-resistant *E. coli* strain TPR 201 according to methods described by Traub *et al.*^{9,11}.

Figure 1 shows the kinetics of methylation with kasugamycin-resistant core particles as a substrate; 16S RNA is not a substrate for methylation. The extent of methylation of core particles is comparable with that reported by Helser *et al.*⁹. Subsequently, 30S ribosomes were reconstituted from CsCl core particles and split proteins (SP30) under the conditions used for total reconstitution of 30S ribosomes^{11,12}. The resulting particles were assayed for methylation without isolation. As Fig. 2 shows, addition of split proteins to produce 30S ribosomes resulted in an inhibition of methylation.

These results indicate that 16S RNA is not a substrate for methylation and that binding of some ribosomal proteins is essential for methylation to occur. Completion of assembly of 30S ribosomes must result in a structure in which the AACUG sequence is in an unreactive state for methylation.

To determine which proteins are involved in conferring substrate specificity to 16S RNA, we studied the methylation of 16S RNA in ribonucleoprotein complexes with different protein compositions. Purified 30S proteins were mixed in three groups as described in the legend to Table 1. These three groups included all the proteins present in the core particles as well as S5 (according to the accepted nomenclature of Wittmann *et al.*¹³. S5 was included in the mixture to ensure efficient binding of S21 (Fig. 4)¹⁴.

16S RNA was incubated with groups of proteins under reconstitution conditions and the mixture was assayed for methylation of the resulting ribonucleoprotein complexes as described previously. Table 1 shows that all three groups of proteins (A, B, and C) were required for methylation of 16S RNA (Table 1a, line 8). Omission of any group gave a non-methylatable complex (Table 1a). In addition, the results indicate that the formation of an RNA-protein complex which can be methylated is temperature-dependent. Incubation at 0° C and assay for methylation at 37° C resulted in reduced methylation (Table 1b). The observed low level of methylation was presumably due to some reconstitution during the assay at 37° C.

Several single-component omission experiments were done to determine whether all proteins in each group were essential for methylation (Table 2). Omission of S13 and

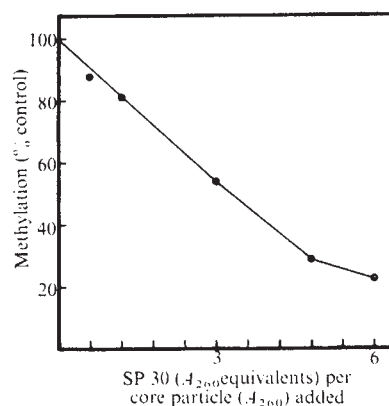


Fig. 2 Inhibition of methylation after reconstitution of 30S ribosomes from core particles and split proteins. CsCl core particles and split proteins (SP30) were prepared as described by Traub *et al.*¹¹. The split proteins (SP30) were dialysed against TRI buffer (30 mM Tris-HCl, pH 7.4, 20 mM MgCl_2 , 6 mM 2-mercaptoethanol and 1 M KCl). 30S ribosomes were reconstituted under 'reconstitution conditions' as described in Fig. 1 and the resulting particles were methylated essentially as described in the legend to Fig. 1. One A_{260} equivalent of SP30 corresponds to the split proteins obtained from 1 A_{260} unit of 30S ribosomes¹¹. 100% methylation corresponds to 3.3 methyl groups per core particle.

S20 from group A did not affect methylation but the omission of any one of the proteins S4, S8, or S15 caused a reduction in methylation (Table 2a). Addition of S4, S8, and S15 in combinations of two to groups B and C did not bring about methylation (Table 2d). Thus, three of the proteins (S4, S8 and S15) in group A are needed for methylation (Table 2d, line 4). Single-omission experiments (Table 2b) suggest that only S16 of group B is required for methylation. In the presence of groups A and C, however, addition of S16 only was not sufficient for maximum stimu-

Table 1 Reconstitution of core structure for methylation

		c.p.m.	%
(a)	(1) 16S	1,047	15
	(2) 16S + Gp A	1,406	21
	(3) 16S + Gp B	1,046	15
	(4) 16S + Gp C	918	14
	(5) 16S + Gp A + Gp B	1,916	29
	(6) 16S + Gp A + Gp C	2,073	31
	(7) 16S + Gp B + Gp C	1,340	20
	(8) 16S + Gp A + Gp B + Gp C	6,641	100
	(9) Core particle	8,100	122
(b)	16S + Gp A + Gp B + Gp C	0° → 30'	2,563 39

Proteins in various groups are: A, S4, S8, S20, S15 and S13; B, S7, S16, S17, S19, and S5; C, S6, S18, S11, S21 and S12. 30S ribosomal proteins were purified from *E. coli* Q13 as described by Held *et al.*¹² Purified ribosomal proteins were dialysed against reconstitution buffer containing 0.5 M KCl. Particles of indicated protein composition were reconstituted by incubating 0.25 A_{260} units of kasugamycin-resistant 16S RNA with a 2 molar excess of pure proteins. Reconstitution and methylation assays were as described previously. In the experiment in (b), 16S RNA was incubated in the reconstitution buffer with the indicated proteins at 0° C for 30 min and assayed for methylation at 37° C. 100% methylation (6,641 c.p.m.) corresponds to 2.3 methyl groups per 16S RNA. The extent of methylation (100% values) varied from 2.0 to 3.5 methyl groups per 16S RNA in different experiments. This variation may reflect differences between batches of 16S RNA and proteins used for reconstitution as well as variability of the components of the methylase reaction.

lation of methylation. Both S16 and S17 were required (data not shown, see also below). Thus S16 and S17 are the only proteins needed from group B; S5, S7, and S19 are dispensable.

Single omission of proteins in group C indicate that S6, S11, and S18 are needed for methylation of 16S RNA together with the proteins in groups A and B (Table 2c).

Table 2 Effect of omission of single proteins on the methylation

	% Methylation
(a) (1) 16S	3
(2) Core particle	124
(3) 16S + Gp B + Gp C + Σ Gp A _i	100
(4) — S15	57
(5) — S13	100
(6) — S20	92
(7) — S8	16
(8) — S4	31
(b) (1) 16S + Gp A + Gp C + Σ Gp B _i	100
(2) — S5	101
(3) — S7	104
(4) — S19	111
(5) — S16	36
(6) — S17	101
(c) (1) 16S + Gp A + Gp B + Σ Gp C _i	100
(2) — S21	102
(3) — S6	79
(4) — S18	65
(5) — S12	110
(6) — S11	63
(d) Effect of addition of single proteins to protein mixtures on methylation	
(1) 16S + Gp B + Gp C + S4 + S8	74
(2) + S4 + S15	10
(3) + S8 + S15	15
(4) + S4 + S8 + S15	108

Particles were reconstituted as described previously. The protein composition of groups A, B and C is given in Table 1. In single-omission experiments shown in (a), (b) and (c) the indicated purified proteins were omitted from the reaction mixtures. In (d) the indicated proteins were added to mixtures of group B and group C proteins. 100% methylation corresponds to 2.7 (7,956 c.p.m.), 2.0 (5,976 c.p.m.) and 2.7 (6,596 c.p.m.) methyl groups per 16S RNA for (a), (b) and (c), respectively.

Addition of S6, S11 and S18 individually, or in combinations of two, to groups A and B does not lead to significant methylation (data not presented here). Thus three proteins (S6, S11, and S18) of group C are required for methylation.

These results indicate that at least eight of the 14 proteins present in the core particle (S4, S8, S15, S16, S17, S18, S11 and S6) are necessary for methylation of 16S RNA. Table 3 shows that a methylatable substrate can be formed by addition of this set of eight proteins to 16S RNA. The extent of methylation is almost the same as that observed with particles reconstituted with a mixture of all the core proteins (Table 3, line 5). Thus, the interaction of 16S RNA with eight proteins is needed to form a minimum structure required for methylation. Omission of any one of the proteins in the minimum structure leads to reduced methylation. Omission of S4, S8, S16 or S17 leads to a more drastic reduction in methylation than other proteins.

Inhibition of methylation

As pointed out earlier, Fig. 2 shows that reconstitution of 30S ribosomes from CsCl core particles and split proteins results in inhibition of methylation. To determine whether one or more of the 'split' proteins may be involved in the inhibition, we performed several single component

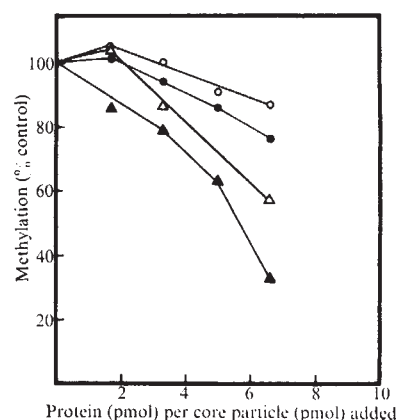


Fig. 3 Effect of single protein omission on methylation of reconstituted 30S ribosomes. *E. coli* A19 salt-washed ribosomes prepared as described in the text were dissolved in TMAI and sedimented through 30% sucrose in TMAI. The ribosomal subunits were separated by zonal ultracentrifugation in a Ti 15 rotor (5–30% sucrose gradient in 10 mM Tris-HCl, pH 7.4, containing 0.3 mM $MgCl_2$, 30 mM NH_4Cl and 6 mM 2-mercaptoethanol). 30S ribosomal proteins were extracted and purified essentially as described by Hardy *et al.*²⁴ The purified split proteins omitted from reconstitution mixtures are indicated in the figure. Reconstitution and assay conditions are as previously described. 100% methylation corresponds to 3.3 methyl groups per core particle. ○, minus S3; ●, minus S14(S9); △, minus S10; ▲, minus S1; □, minus S2 (S1, S5).

omission experiments in which each one of the 'split' proteins (S1, S2, S3, S5, S9, S10 and S14) was omitted from reconstitution mixtures^{7,11}.

Figure 3 shows that omission of S1, S2 or S5 did not affect inhibition of methylation by the rest of the split proteins. Omission of S3, S9 or S14 resulted in a considerable reduction in the inhibition of methylation and omission of S10 partially reduced the inhibition. Addition of

any one of the purified split proteins to the core particles does not inhibit methylation (data not shown here). It should be noted that the extent of inhibition observed in experiments with purified split proteins was somewhat less than with unpurified split proteins (data in Fig. 2).

We next asked if the inhibitory proteins S3, S9, S10 and S14 are sufficient to inhibit methylation of the minimum methylatable structure established above (Table 3). Three proteins (S7, S19 and S5), not required for methylation, were also added since they are necessary for the binding of proteins S3, S9, S10 and S14 (Fig. 4)¹⁴. Methylation was not inhibited when the inhibitory proteins were added together with S7, S19 and S5 (Table 4, line 3). The presence of the additional proteins may have stabilised the minimum structure and thus stimulated the reaction.

Table 3 Establishment of minimum core structure for methylation

	% Methylation
(1) 16S	9
(2) 16S + Gp A + Gp B + Gp C	100
(3) 16S + Gp I	15
(4) 16S + Gp II	11
(5) 16S + Gp I + Gp II	89
(6) — S4	13
(7) — S8	14
(8) — S16	17
(9) — S17	19
(10) — S15	33
(11) — S18	42
(12) — S11	42
(13) — S6	44

Proteins present in group I are S4, S8, S16 and S17; and Group II, S15, S18, S11 and S6. 100% methylation corresponds to 2.2 (6,576 c.p.m.) methyl groups per 16S RNA.

Thus, in the experiment shown in Table 4 (line 3) methylation was not inhibited by the inhibitory proteins even though all the proteins present in core particles were present with the exception of S20, S21, S13 and S12. Addition of each one of these four proteins to the mixtures did not result in inhibition (Table 4, lines 4–7), but addition of S20 and S21 together resulted in a significant inhibition of methylation (Table 4, line 9). S12 slightly enhanced the inhibition observed with S20 and S21 (Table 4, line 13).

Our results can be summarised as follows. (1) 16S RNA is not methylated but CsCl core particles are active substrates. (2) Interaction of eight proteins (S4, S6, S8, S11, S15, S16, S17 and S18) with 16S RNA results in the formation of a minimum structure which can be methylated. (3) Addition of split proteins to core particles leads to an inhibition of methylation. Proteins S3, S9, S10 and S14 are the split proteins responsible for inhibition. S1, S2 and S5 are not involved in inhibition. (4) In addition to the inhibitory split proteins, the inhibition of methylation of 16S RNA is observed only in the presence of most of the core proteins, several of which are not essential for methylation (that is S7, S19, S20 and S21).

Methylation and assembly of 30S ribosomes

In the experiments reported here, the 30S ribosomal proteins required for *in vitro* methylation of 16S RNA, as well as those involved in inhibition of this reaction, have been identified. Table 3 shows that eight 30S protein (S4, S8, S15, S16, S17, S18, S11 and S6) are required for methylation of the AACUG sequence in 16S RNA *in vitro*.

Six 30S proteins (S4, S7, S8, S15, S20 and S17) have been shown to interact with 16S RNA directly in the absence of other proteins in a site-specific manner^{14–19}. Four of these proteins, S4, S8, S15 and S17, are necessary for *in vitro* methylation of 16S RNA. Zimmermann *et al.* reported that S4, S8 and S15 (as well as S20 and possibly S13) interact specifically with an RNA fragment toward the 5' half of the 16S RNA²⁰. The m⁶A m⁶A CCUG sequence is about 25 nucleotides from the 3'-OH end of 16S RNA²¹. Thus, several proteins (S4, S8 and S15) which are known to bind some distance from the site of methylation are nonetheless necessary to establish an RNA-protein structure which can be methylated. This suggests that these proteins are required for a structural change which affects the substrate specificity of the 3'-OH end of the RNA for methylation. It is interesting that S7, which is the only protein known to bind directly near the 3'-OH end of 16S RNA²⁰, and hence close to the methylation site, is not required for methylation (data described in Table 3).

A comparison of the proteins required for methylation of 16S RNA and the assembly map (Fig. 4)^{14,16} indicates these proteins are strongly interrelated and occupy an early part of the map. In addition to S4, S8, S16, and S17, the group of interrelated proteins shown on the left side of the assembly map (S15, S18, S6 and S11) are required for methylation while all of the proteins on the right side of the map whose binding strongly depends on the prior binding of S7, are not required. This latter group of proteins, however, inhibits methylation (that is S9, S14, S10 and S3). It is probable that *in vivo* the methylating enzyme acts on intermediate particles which contain the essential eight proteins and perhaps other proteins, but lack some or all of the inhibitory proteins.

Analysis of the protein composition of ribosomal precursor particles is consistent with the early incorporation of the eight proteins required for methylation during *in vivo* assembly. 21S particles accumulated at low temperatures by a cold-sensitive mutant of *E. coli* contain all the

Table 4 Inhibition of methylation of minimum core structure

	% Methylation
(1) 16S	19
(2) 16S + Gp I + Gp II	100
(3) 16S + Gp I + Gp II + Gp III	125
(4) + S20	113
(5) + S12	129
(6) + S21	116
(7) + S13	132
(8) + S20 + S12	100
(9) + S20 + S21	65
(10) + S20 + S13	104
(11) + S20 + S12 + S13	97
(12) + S20 + S13 + S21	68
(13) + S20 + S12 + S21	54
(14) + S12 + S13	120
(15) + S12 + S21	99

The protein composition of groups I and II are the same as that given in Table 3. Proteins present in group III are S7, S19, S3, S5, S9, S10 and S14. 100% methylation corresponds to 3.5 (10,287 c.p.m.) methyl groups per 16S RNA.

proteins found to be required for methylation with the exception of S11²². In addition to the set of seven proteins, these particles also contain proteins which are not required for methylation (S7, S19, S20, S13 and possibly S9). It should be pointed out that these *in vivo* particles contain precursor 16S RNA which is not methylated. Five of the eight proteins required for methylation (S4, S8, S15, S16 and S17) are also present in the *in vivo* 22S particles studied by Nierhaus *et al.*²³.

Size and location of the transforming region in human adenovirus type 5 DNA

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Fragments of human adenovirus type 5 DNA as small as one million daltons can transform cells in vitro. The DNA segment which induces transformation is located between 1 and 6% from the left end of the Ad 5 DNA molecule.

HUMAN adenoviruses contain linear double stranded DNA of molecular weight 20×10^6 to 25×10^6 (refs 1, 2). They can be classified into three subgroups: A, B, and C which vary in their degree of oncogenicity³. Viruses of subgroup C (to which adeno 5 belongs) are nononcogenic in that they do not give rise to tumours after injection into newborn hamsters⁴; they can, however, transform cells *in vitro*^{5,6} and in certain conditions the transformed cells can induce tumours in animals^{7,8}.

We have recently shown that Ad 5 DNA is infectious for human KB cells⁹ and that it can transform rat cells *in vitro*¹⁰. Although DNA from simian adenovirus 7 had previously been reported to be oncogenic in newborn hamsters¹¹ our observations provided the first demonstration of transformation *in vitro* by adenovirus DNA. The development of an *in vitro* assay¹⁰ afforded the opportunity for quantitative studies of transformation by adenovirus DNA.

While examining the effects of various treatments on the biological activity of Ad 5 DNA we observed that transforming activity was much more resistant to shearing than was infectivity: DNA preparations which were rendered noninfectious by shearing still retained undiminished transforming activity. We concluded that we might be observing transformation by DNA fragments. The studies presented here were undertaken to prove that Ad 5 DNA fragments of less than genome size have transforming activity, to determine the minimum size of fragments which retain the ability to transform cells, and to map the location of the transforming (T) segment within the viral DNA molecule.

The assays for biological activity were carried out using a modification of what we now call the calcium technique⁹. This method is based on the formation of a coprecipitate of DNA and calcium phosphate (formed by adding CaCl_2 to solutions of DNA in phosphate-containing buffer) which on addition to cell cultures becomes associated with the cells. DNA uptake then takes place at 37°C by a process which again requires calcium ions. The most important modification which we have introduced in the technique is the addition of a carrier DNA to all assays. This was shown to improve the efficiency of the assay⁹ and has the important advantage of producing a linear dose response (ref. 12 and Fig. 1), thus simplifying quantitative calculations of specific activities.

Transformation by sheared Ad 5 DNA

Ad 5 DNA which was sheared to fragments having a sedimentation coefficient ($S_{20,w}$) of 22S (molecular weight 8×10^6) had the same transforming activity as unsheared DNA (Fig. 1) yet infectivity of the sheared DNA could not be detected when

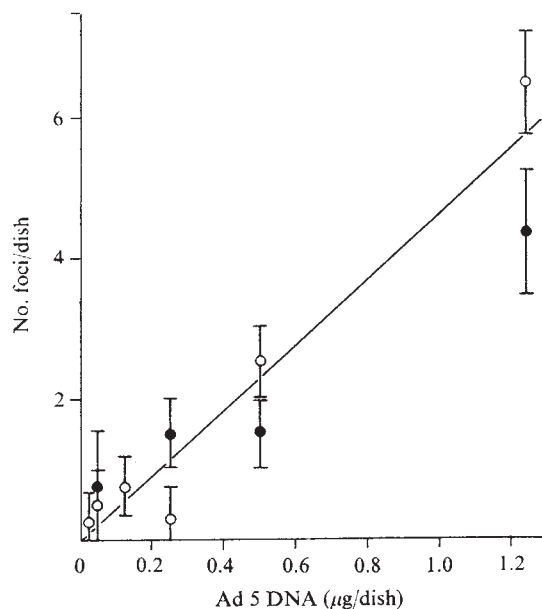


Fig. 1 Dose response for transformation of primary rat kidney cells by intact (○) and sheared (●) Ad 5 DNA. The DNA was extracted from purified Ad 5 as described previously¹. Shearing was carried out by passing $200 \mu\text{g ml}^{-1}$ solutions of Ad 5 DNA in $0.1 \times \text{SSC}$ (15 mM NaCl, 1.5 mM sodium citrate) 10 times through a needle (0.72 mm inside diameter $\times$ 30 mm long) using maximum hand pressure on a syringe with a 15 mm diameter plunger. All sedimentation coefficients were determined by band sedimentation in 1 M NaCl at 20°C at an initial DNA concentration of approximately $25 \mu\text{g ml}^{-1}$ in an analytical ultracentrifuge equipped with ultraviolet optics and a photoelectric scanner. Coefficients have been corrected for viscosity but not for concentration dependence. Intact Ad 5 DNA sedimented at 30S and DNA sheared as described above sedimented at 22S. The assays for transforming activity were based on previous studies on infectivity⁹ and transforming activity¹⁰ of Ad 5 DNA and were carried out on subconfluent cultures of primary rat kidney cells prepared by trypsin dispersion of kidneys from week old Wistar rats. Viral DNA was diluted into HEPES buffered saline (HeBS: 8.0 g l^{-1} NaCl, 0.37 g l^{-1} KCl, 0.125 g l^{-1} $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 1.0 g l^{-1} dextrose, 5.0 g l^{-1} N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid, final pH 7.05) containing salmon sperm DNA at $10 \mu\text{g ml}^{-1}$. CaCl_2 was added to 125 mM and the mixture was incubated at room temperature for 10–20 min during which time a precipitate formed. Aliquots (0.5 ml per dish) of the resulting suspension (consisting of a co-precipitate of DNA and calcium phosphate) were then added to rat kidney cells growing in 60 mm plastic Petri dishes containing 5 ml Eagle's minimum essential medium supplemented with 10% calf serum (MEM+10% CS) and the dishes were placed in a 37°C , 5% CO_2 incubator. After 3–4 h at 37°C the medium was removed and replaced with fresh MEM+10% CS and the incubation at 37°C continued. After 3–4 d the medium was replaced with low calcium medium⁹ (MEM for suspension cultures+0.1 mM CaCl_2 +5% CS) and this was refreshed at 3–4 d intervals. At 3 weeks, by which time few normal cells remained attached to the dishes, the cultures were fixed, stained with Giemsa, and transformed colonies were counted. Each point represents the average of 4 dishes and the error bars represent ± 1 s.d.

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assayed in conditions which resulted in 20–30 PFU μg^{-1} for intact DNA. Furthermore, permissive cells (hamster and human) which could not be transformed by intact DNA (due to the resulting productive infection which spread to all the cells in the culture) could be transformed by noninfectious sheared DNA. These observations showed that transformation did not require infectious DNA molecules and suggested that DNA fragments smaller than a viral genome had the ability to transform. They did not, however, eliminate the possibility that transforming activity was associated with a shear-resistant (for example, circular) molecular form of Ad 5 DNA. To rule out this possibility intact and sheared DNA preparations were fractionated through sucrose gradients and fractions were assayed for transforming activity.

Activity should sediment at the same rate before and after shearing if it were associated with a shear resistant form of Ad 5 DNA. The results shown in Fig. 2 clearly demonstrate that this was not the case. Transforming activity of unsheared preparations sedimented with the same velocity as intact DNA and

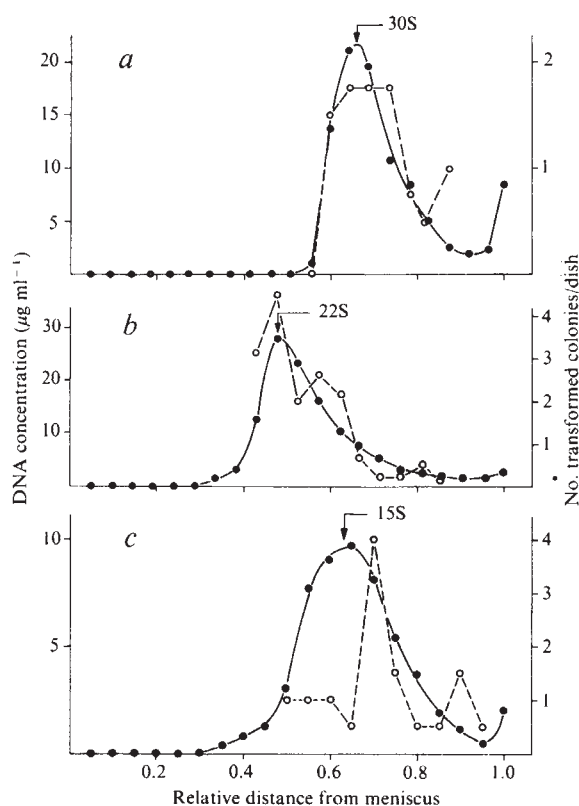


Fig. 2 Transformation by intact and sheared Ad 5 DNA fractionated by velocity sedimentation. Aliquots of ^3H -Ad 5 DNA (2,000 c.p.m. μg^{-1}) were layered on to 5–22% exponential sucrose gradients containing 1 M NaCl, 0.01 M Tris, pH 8.1, and 0.5 mM disodium ethylenediaminetetraacetate which had been prepared by pumping sucrose solutions from a 70 ml mixing chamber connected to a reservoir containing 50% sucrose. Gradients were centrifuged at 4°C for 22 h (a and b) or 34 h (c) at 25,000 r.p.m. in a SW 27 rotor and fractionated from the top. DNA concentrations were determined by counting 25 μl and transforming activity was measured as described in the legend to Fig. 1 (50 μl DNA was assayed per dish). Recovery of radioactivity was 60–70%. The sedimentation coefficients given in the figure were determined by band sedimentation in the analytical ultracentrifuge before the DNA was fractionated through sucrose gradients. a, Intact DNA. Transforming activity was measured in two separate experiments, two dishes/fraction. The points therefore represent the mean of four dishes. b, DNA sheared to 22S according to the legend to Fig. 1. Each point represents the mean of two separate assays on two dishes each. c, DNA sheared by passage 20 times through a 0.42 mm $\times$ 22 mm long needle using maximum hand pressure on a syringe with a 9 mm plunger. Fractions were assayed once for transformation, 2 dishes per assay. ●, DNA concentration; ○, Transforming activity.

shearing reduced the sedimentation velocity and transforming activity of the DNA to the same extent (Fig. 2a and b). The results shown in Fig. 2c indicate that even DNA fragments smaller than 15S (3×10^6 daltons) could transform with an efficiency as high as that of intact DNA. Thus transforming activity appeared to be retained even after breaking the DNA to fragments as small as 1/8 of the viral genome. It was now of considerable interest to determine more precisely the degree to which Ad 5 DNA could be fragmented before all transforming activity was lost.

Minimum size of transforming fragments

If transformation by Ad 5 DNA required the integrity of some segment (the T segment) of length L then transforming activity of randomly fragmented DNA should decrease as the fragment size approached L and should be zero for fragments less than or equal to L in molecular weight. To obtain an estimate of L, Ad 5 DNA was extensively fragmented by shearing or sonication (resulting in modal sedimentation coefficients of 11.5S and 12S, respectively), fractionated by velocity sedimentation, and assayed for transforming activity.

The results, plotted in Fig. 3a and b, showed that for both sheared and sonicated preparations, transforming activity was found only in fractions containing DNA fragments sedimenting faster than 10S. To ensure that the molecular weight of the DNA fragments had not changed between the time they were prepared and the time they were assayed for transforming activity, and to determine as accurately as possible the molecular weight of DNA fragments in the region where transforming activity approached zero, peak fractions from the gradients in Fig. 3a and b were concentrated after transforming activity had been measured and sedimentation coefficients were redetermined in the Model E ultracentrifuge. The resulting values (shown in Fig. 3a and b, and differing only slightly from the modal values obtained before fractionation) were then used to calibrate the gradients to determine molecular weights from the equations in the legend to Fig. 3.

The graph of specific transforming activity plotted versus molecular weight in Fig. 3c shows that fragmentation of Ad 5 DNA, whether by shearing or sonication, began to reduce transforming activity when the fragment size fell below about 2×10^6 daltons and activity was completely abolished at slightly below 1×10^6 daltons. If loss of activity at this point was due to the introduction of breaks into the T segment then the size of the T segment must be approximately 10^6 daltons. But since there may be other explanations for absence of transforming activity below 10^6 daltons (for example, DNA fragments smaller than 10^6 daltons may be inherently lacking in any biological activity in our assay system) we can only conclude that the T segment is not greater than about 10^6 daltons in length.

The possibility that transformation by small fragments required the interaction of two or more DNA segments can be ruled out since such a mechanism would result in a dose response of 2nd or higher order. Yet we observed no significant departure from a first order dose response with decreasing fragment size. It would appear, therefore, that only one small segment of the Ad 5 DNA molecule is required for transformation and that the molecular weight of this segment is not more than about 10^6 daltons.

The properties of cells transformed by small DNA fragments are now under investigation. We can only say that such transformed cells do not appear to differ morphologically from those transformed by intact DNA. One line of rat kidney cells transformed by fragments of molecular weight 1.5×10^6 is now at the 27th passage in culture; several transformed hamster lines (transformed by DNA fragments of molecular weight about 8×10^6) have been maintained in culture for 5–23 passages and one transformed human cell line is now at passage 51. Furthermore, of five transformed hamster cell lines tested for tumorigenicity, all induced tumours when injected into newborn hamsters. Thus DNA fragments seem to be capable of inducing

not only stable, but also oncogenic transformation. By indirect immunofluorescence using serum from tumour-bearing hamsters, we have demonstrated T antigen in all of several DNA-transformed cell lines tested, including one transformed by fragments of molecular weight 1.5×10^6 . (The serum was obtained from hamsters bearing tumours induced by Ad 5 transformed cells and was pretested against transformed rat cells which had previously been shown positive for T antigen¹⁰.)

Location of the T segment

The finding that fragments of Ad 5 DNA had the ability to transform cells suggested the feasibility of mapping the T segment

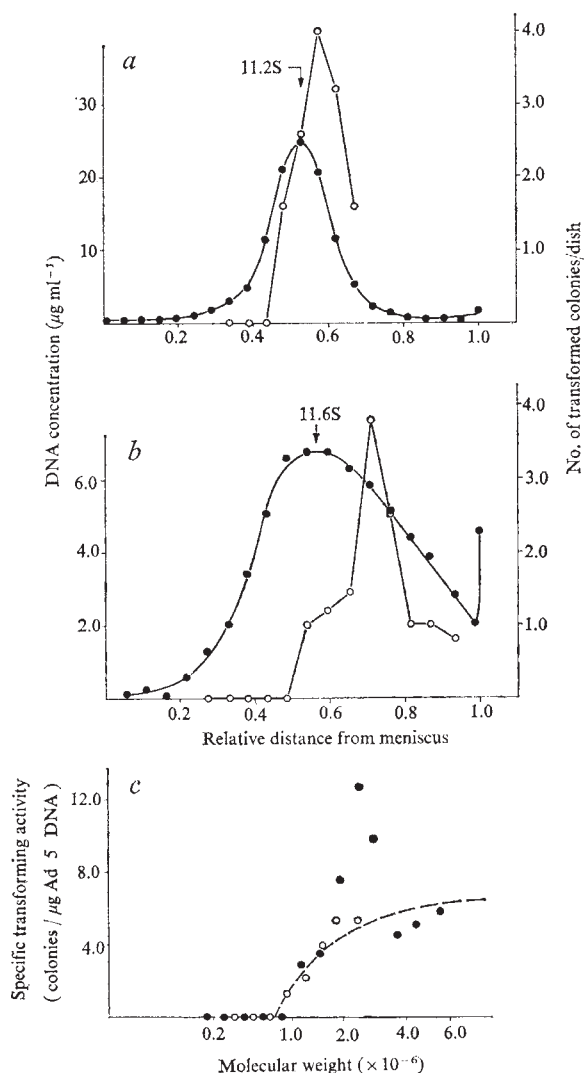


Fig. 3 Transformation by extensively fragmented Ad 5 DNA. *a*, DNA at $200 \mu\text{g ml}^{-1}$ was sheared for 3 min in a Sorvall omnimixer micro attachment by a stainless steel blade rotating at 40,000 r.p.m. A sedimentation coefficient of 11.5S was determined for this DNA before it was fractionated on sucrose gradients. DNA was sedimented by centrifugation at 23,000 r.p.m. for 44 h on gradients prepared according to the legend to Fig. 2. After fractionating and assaying aliquots for transforming activity (50 µl per dish) the peak fraction was concentrated and the sedimentation coefficient again measured to give the value shown in the figure. *b*, DNA at $100 \mu\text{g ml}^{-1}$ was sonicated in a MSE sonicator for 15 s, at 20,000 c.p.s., amplitude 2 µm. This resulted in a DNA preparation which sedimented in the analytical ultracentrifuge as a rather broad band having a modal sedimentation coefficient of approximately 12S. (The coefficient shown in the figure was determined for the combined fractions 10 and 11 after assaying for transformation.) ●, DNA concentration; ○, transforming activity, mean of 5 dishes per point. *c*, Specific transforming activity of Ad 5 DNA fragments calculated from panels A (○) and B (●) as a function of molecular weight. Molecular weights were calculated from the equations $D = kS$ and $S = 0.0882 M^{0.346}$ (ref. 13) where D is distance sedimented and k was determined from the sedimentation coefficients shown in panels A and B.

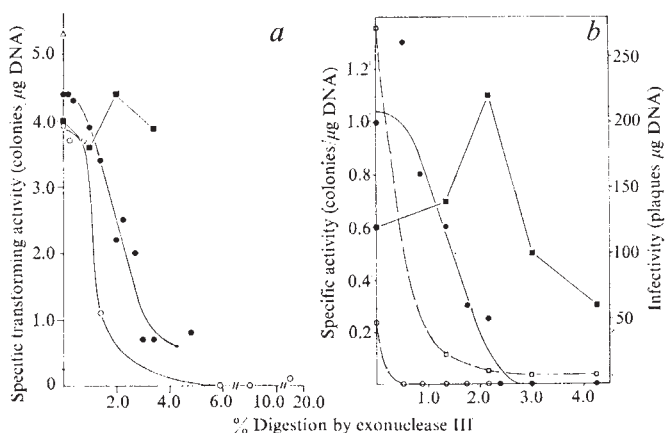


Fig. 4 *a*, Transforming activity of Ad 5 DNA after treatment by exo III or exo III + S1. Enzymatic digestions were carried out in the following way: To 1 ml ^3H -Ad 5 DNA (10^4 c.p.m. μg^{-1} , 300–500 $\mu\text{g ml}^{-1}$ in $0.1 \times \text{SSC}$) was added 0.1 ml of a buffer containing 33 mM each of Tris, pH 8.0, MgCl_2 , and 2-mercaptoethanol. Digestion was initiated by the addition of 0.1 ml exonuclease III (2000 U ml^{-1} , 1.5×10^5 U mg^{-1} in 40 mM potassium phosphate buffer, pH 6.5, containing 1 mM 2-mercaptoethanol and 0.4 M KCl) and incubation at 20°C . Exonuclease III was prepared as described²³ with additional purification by phosphocellulose chromatography and Sephadex G100 gel filtration²⁴. The extent of digestion was monitored by removing 10 µl aliquots and determining the fraction of acid soluble radioactivity. At various times 80 µl aliquots were removed and exonucleolytic digestion was stopped by adding 20 µl 0.15 M sodium acetate buffer, pH 4.2, containing 5 mM ZnSO_4 and 1.5 M NaCl. Single-stranded ends were subsequently degraded by adding 20 µl single-strand specific nuclease S1 from *Aspergillus oryzae* (300 U ml^{-1} , 1.5×10^5 U mg^{-1} , in 20 mM sodium acetate buffer, pH 4.6, containing 0.1 mM ZnSO_4 , 0.3 M NaCl, and 5% glycerol). S1 purification was essentially as described by Vogt²⁵. The reaction was carried out at 37°C for 30 min and was terminated by the addition of 10 µl 0.1 M EDTA, pH 8.0. (The % digestion by S1 equalled the amount of DNA made acid soluble by exo III.) Finally, HeBS containing 0.5 mM EDTA was added to give a final DNA concentration of 50 $\mu\text{g ml}^{-1}$ and the samples were dialysed against HeBS + 0.5 mM EDTA. Assays for transforming activity were carried out according to the legend to Fig. 1, 2 µg DNA per dish, 5 dishes per point. (△) Untreated DNA; (■) exo III treated DNA; (○, ●), exo III-S1 treated DNA. *b*, Infectivity and transforming activity of Ad 5 DNA treated with exo III or exo III + S1. Enzyme digestions were carried out as in *a* except that unlabelled Ad 5 DNA was used and the extent of digestion by exo III was determined by measuring the amount of deoxynucleotides incorporated into the treated DNA by DNA polymerase I (purified from an *E. coli* pol A mutant deficient in 5'-3' exonucleolytic activity²³). Transforming activity (■, ●) and infectivity (□, ○) were assayed as described in the legend to Fig. 1 except that infectivity assays were carried out on primary human embryonic kidney (HEK) cells or on a line of HEK cells transformed by Ad 5 DNA. ■, □, exo III treated DNA; ●, ○, exo III + S1 treated DNA.

within the viral DNA molecule and of purifying this segment. The first step which we took in this direction was to determine which half of the molecule contained transforming activity. Since the DNA of Ad 5 (like that of Ad 2 (refs 14, 15)) has an asymmetric distribution of GC, it was possible to separate the two halves of Ad 5 DNA by equilibrium buoyant density centrifugation of half molecules in a $\text{Hg(II)}\text{-Cs}_2\text{SO}_4$ gradient according to the method of Nandi, Wang and Davidson¹⁶. DNA was sheared, fractionated on sucrose gradients to purify the halves which were then separated in a Cs_2SO_4 gradient in the presence of Hg(II) . Fractions corresponding to AT or GC-rich halves were pooled, dialysed against HeBS, and aliquots were analysed by buoyant density centrifugation in CsCl in a Model E analytical ultracentrifuge. The results indicated that a single fractionation on a $\text{Hg(II)}\text{-Cs}_2\text{SO}_4$ gradient was sufficient to provide relatively pure preparations of AT-rich and GC-rich halves (the cross contamination was less than about 10%). When these preparations were assayed for transforming activity it was found that the AT-rich halves had less than 5% of the transforming activity observed for the GC-rich fractions. (0.7 colonies per µg AT-rich

Table 1 Calculation of the location of the T segment

Experiment	Fraction of intact single strands	Average no. nicks/strand	Effective no. nicks/strand introduced by exo III*	No. 3' ends per molecule†	% exo III digestion resulting in 50% reduction in transforming activity	Total % digestion by exo III followed by S1 at 50% reduction	Total % digestion per 3' end (distance from left end of Ad 5 genome to T segment)‡
1	70%	0.36	0.1	2.92	1.3	2.6	0.89
2	32%	1.14	0.1	4.48	2.2	4.4	0.98
3	100%	0	0.1	2.2	1.4	2.8	1.27

The fraction of intact single strands was determined by band sedimentation in alkali in the analytical ultracentrifuge. The average number of nicks per strand was calculated from this fraction assuming a Poisson distribution. The % digestion by exo III followed by S1 was double that by exo III alone.

* The exonuclease preparation had a low level of contaminating endonuclease activity introducing nicks which could then serve as substrates for exonuclease digestion.

† The number of 3' ends per molecule is 2 + the number of nicks per molecule.

‡ Calculated from the total % digestion divided by the number of 3' ends per molecule.

DNA compared to 18 colonies μg^{-1} for the GC-rich halves.) Thus the T segment appeared to be located in the GC-rich or left half of the Ad 5 DNA molecule, a finding which has been recently confirmed by studies on the transforming activity of DNA fragments obtained by treatment with the R1 restriction endonuclease from *E. coli*.

A more precise localisation of the T segment was obtained by assaying transforming activity of Ad 5 DNA treated with *E. coli* exonuclease III (exo III) (which removes mononucleotides from the 3' end of polynucleotides in duplex molecules¹⁷) followed by single strand specific nuclease S1 from *Aspergillus oryzae*. The combination of these two enzymatic treatments has the effect of digesting the Ad 5 DNA molecule inward from the two ends and should result in a decrease in transforming activity when the digestion reaches the region required for transformation.

The results of two experiments are illustrated in Fig. 4a. Treatment of Ad 5 DNA with either exo III or S1 separately did not significantly affect transforming activity. The combination of exo III and S1 treatment initially had no effect on transforming activity but after an exo III digestion of about 1% (that is, a total digestion of 2% by the two enzymes), transforming activity dropped rapidly. In contrast, infectivity of Ad 5 DNA was immediately eliminated by exo III-S1 digestion (Fig. 4b); digestion of as little as 0.2% (data not shown) resulted in a complete loss of infectivity (> 200-fold decrease) indicating that all molecules were digested by the enzymatic treatment. Figure 4b shows also that infectivity was reduced by exo III alone, an effect which is presently being investigated in more detail to determine if, for example, it is related to the inverted terminal repetition contained in adenovirus DNAs^{18,19}. The fact that transformation was not immediately affected by exo III-S1 treatment clearly shows that the extreme ends of the Ad 5 DNA molecule are not required for transformation.

We interpret the eventual loss of transforming activity after treatment of Ad 5 DNA with exo III and S1 as being the result of digestion into the T segment and in Table 1 have used the data of Fig. 4 to calculate the distance from the end of the Ad 5 DNA molecule to the beginning of the T segment. When account has been taken for single strand nicks (which are starting points for exonucleolytic digestion) the results from three experiments are in good agreement and give a mean value of 1.1%. From the fact that transforming activity was located on the GC-rich half of the Ad 5 DNA molecule, and from the results of Fig. 3 we conclude that the T segment begins at 1% from the left end of the Ad 5 genome and terminates at about 5–6%.

Implications

Transformation by DNA tumour viruses is often considered to be analogous to lysogenisation of bacteria by bacteriophage, a process thought to involve the integration of the phage genome into the host chromosome by a reciprocal recombination between the host DNA and a circular form of the phage DNA²⁰. The results presented in Figs 2 and 3 provide clear evidence that

Ad 5 DNA fragments of considerably less than genome size have the ability to transform cells. As it is rather unlikely that such randomly generated fragments can circularise, these results suggest that transformation does not require a circular viral DNA molecule. That fragments as small as 10^6 daltons can transform cells also implies that the proportion of the viral genome which is required to initiate and maintain transformation is less than about 5%. This is in reasonable agreement with results of attempts to determine the size of the transforming region by radiation studies²¹, and is consistent with the fact that only about 4–10% of the viral genome is transcribed in rat cells transformed by Ad 2 (ref. 22). Thus, the T segment is large enough to code only for 1–2 average-sized proteins so that presumably not more than about two viral genes are involved in transformation by Ad 5 and these must be contiguous.

We have localised the T segment between 1 and 6% from the left end of the Ad 5 genome. Several lines of evidence are in agreement with this finding. First, Gallimore (P. H. Gallimore, personal communication) has shown that the left end of the Ad 2 genome from 0–14% was always present in several lines of Ad 2 transformed cells. Second, preliminary results indicate that treatment of Ad 5 DNA with two restriction enzymes which cleave in the region between 1 and 6% from the left end results in loss of transforming activity while treatment with a third enzyme which cleaves just to the right of this region leaves transforming activity undiminished and activity appears to be associated with the fragment originating from the left end of the Ad 5 DNA molecule (A. J. van der E., C. Mulder and F. L. G., unpublished).

As in the case of transformation by intact viruses, the mechanism by which the T segment induces cell transformation remains to be elucidated. However, the purification of the T segment, which now appears to be a possibility, should provide a simpler system with which to study the genes and gene products involved in oncogenic transformation.

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letters to nature

Photoelectric photometry of OH471 and OQ172, and other optical objects near radio sources

IDENTIFICATION of the optical objects associated with radio sources is usually made by using the blue and red photographs of the National Geographic Society–Palomar Observatory Sky Survey (PSS). Objects lying near the radio positions are generally counted as possible identifications if they appear diffuse and are therefore galaxies, or appear stellar and blue, and are therefore quasi-stellar object (QSO) candidates. Only if radio positions of $\lesssim 1''$ accuracy are available can identifications be made with red or neutral stellar objects without gross contamination of the true identifications by foreground galactic stars, most of which appear neutral or red on the PSS prints.

Among radio source identifications hitherto made using accurate ($1''$) positions there are few red or neutral stellar objects compared with the number of blue ones¹, but they include some of the most interesting of the identifications. They include: *a* QSOs with redshifts $z \gtrsim 3$, where $\text{Ly}\alpha$ emission is redshifted to $\lambda \gtrsim 5,000 \text{ \AA}$ and there are numerous absorption lines blueward of $\text{Ly}\alpha$, both effects combining to make the object appear redder than QSOs with lower redshifts; *b* galaxies, which are so compact as to appear stellar on the PSS plates; and *c*, objects like OJ287 which have featureless spectra, often with a redder continuum than those of most QSOs. Objects in classes *a* and *b* are usually recognised once their spectra are obtained since those known in these categories have strong $\text{Ly}\alpha$ emission (class *a*) or strong sharp emission lines of, say, [O II] and [O III] (class *b*). Those in class *c* can only be distinguished from stars on the basis of their featureless spectra which, when the objects are faint, may be a difficult observational task since not all stars show strong absorption lines. In this case, UBV photometry can confirm the identification through the particular colours of these objects.

Radio positions with accuracy $\sim 1''$ are routinely measured at the University of Texas Radio Astronomy Observatory (UTRAO) and a small percentage of the sources have been identified with red or neutral stellar objects¹. Here, I report on measurements of broadband colours for a small group of the Texas identifications (together with OH471, identified using the same techniques²), comparing these with colours of previous radio source identifications. I discuss the increased reddening

of QSOs with increasing redshift. All the identifications are within $3''$ of the accurate radio positions, except for one which is within $5''$ (Table 1).

U , B and V magnitudes were measured with two photometer systems on the 82-inch (2.1-m) Struve reflector of McDonald Observatory. One system employed a selected RCA 1P21 and the other a RCA 4516 (S20 photocathode) phototube. The U , B , V measurements were referred to that system defined by the measurements by Crawford, Golson and Landolt³ and by Landolt⁴. The results of the measurements, and other data, are given in Table 1. Errors given there comprise r.m.s. (random) counting statistical errors added in quadrature to estimated systematic calibration uncertainties. The forms of the spectral energy distributions for these objects are shown by their positions in the $U-B$, $B-V$ diagram (Fig. 1), where the new data are compared with previous photometry for QSOs, N-galaxies, objects of the OJ287 type, and radio galaxies. Here, no corrections have been applied for galactic reddening.

It is of interest to compare QSOs in different redshift ranges: $z \leq 2.5$, $2.5 < z < 3.0$ and $z \sim 3.5$. The QSOs having $2.5 < z < 3.0$ have $U-B > -0.4$ compared with $U-B < -0.4$ for the lower redshift ones, since for $2.5 < z < 3.0$ $\text{Ly}\alpha$ emission occurs predominantly in the B filter and absorption shortward of $\text{Ly}\alpha$ causes a relative decrease of power received in the U band. The QSO of largest redshift among these objects, 4C05.34 ($z = 2.88$, ref. 5) has the least negative $U-B$ and can barely be distinguished from an F star on the basis of broadband colours alone⁶. Although all the radio-emitting QSOs would have been suggested as QSOs from their blue appearance on the PSS prints, a common criterion employed in suggesting QSO candidates^{7–9} has been that $U-B < -0.4$. On this basis, all the objects having $2.5 \leq z < 3.0$ would have been missed. The two QSOs, OQ172 and OH471, having $z \sim 3.5$ (refs 10, 11) have rather larger $B-V$ values than do QSOs of smaller redshift—because of the further redshift of $\text{Ly}\alpha$ into the V passband. Note also the large difference ($\sim 2 \text{ mag}$) in $U-B$ for these two objects at nearly the same z —attributable to the much greater absorption below the Lyman limit for OH471. (Spectrophotometry by Oke¹² is in quantitative agreement with these results.) OQ172 does appear slightly blue on the PSS prints, whereas OH471 is quite red. Thus, on the basis of UBV colours or apparent colours from the PSS, QSOs of $z > 3.0$ will frequently be indistinguishable from main sequence stars, and objects with greater absorption below the Lyman limit will

Table 1 Details of objects examined

Associated radio source	Other name	Identification [¶]	Date* February 1974	Uncorrected			Corrected† weight means			Position difference	Position ref.	Optical spectrum ref.
				<i>V</i>	<i>B-V</i>	<i>U-B</i>	<i>V</i>	<i>B-V</i>	<i>U-B</i>			
0219+428 3C66A	Like OJ287?		16‡	15.14 ± 0.10	0.48 ± 0.03	-0.38 ± 0.10	14.62	0.30	-0.64	0.7	14	—
			20	15.26 ± 0.03	0.52 ± 0.02	-0.45 ± 0.03						
			22	15.18 ± 0.03	0.50 ± 0.02	-0.53 ± 0.03						
			20	16.96 ± 0.08	1.20 ± 0.08	+0.17 ± 0.10	16.56	1.07	+0.07	1.5	UTRAO	0.118 UTRAO
0456-043	4C-04.17	GAL	20	16.96 ± 0.08	1.20 ± 0.08	+0.17 ± 0.10	16.56	1.07	+0.07	1.5	UTRAO	0.118 UTRAO
0642+449	OH471	QSO	22‡	18.49 ± 0.06	1.08 ± 0.10	+1.7 ± 0.4	17.91	0.89	+1.6	2.6	2	3.40 11, 15
0850-034	4C-03.34	RSO	19	17.78 ± 0.05	1.20 ± 0.04	+0.67 ± 0.06	17.37	1.06	+0.57	2.1	1	— UTRAO
1037+302	4C30.19	GAL	20	16.24 ± 0.05	0.98 ± 0.03	+0.16 ± 0.05	16.04	0.91	+0.11	1.3	UTRAO	— UTRAO
1049+616	4C61.20	QSO	22	16.48 ± 0.04	0.18 ± 0.02	-0.70 ± 0.03	16.25	0.10	-0.76	1.7	1	0.422 16
1147+245 OM280	Like OJ287?		16‡	15.74 ± 0.10	0.52 ± 0.03	-0.60 ± 0.10	15.70	0.43	-0.59	4.9	UTRAO	— 15, 16
			19§	15.94 ± 0.05	0.39 ± 0.04	-0.49 ± 0.06						
			20	15.89 ± 0.03	0.50 ± 0.03	-0.55 ± 0.02						
			16‡	15.76 ± 0.10	1.20 ± 0.03	+1.41 ± 0.10	15.55	1.13	+1.36	2.1	UTRAO	— UTRAO
1430+095	OQ050	star	16‡	15.76 ± 0.10	1.20 ± 0.03	+1.41 ± 0.10	15.55	1.13	+1.36	2.1	UTRAO	— UTRAO
1442+101	OQ172	QSO	22	17.78 ± 0.03	0.80 ± 0.02	-0.37 ± 0.03	17.57	0.73	-0.42	0.4	2	3.53 10
1600+148 4C14.61	RSO		19	18.66 ± 0.15	1.70 ± 0.15	—	18.54	1.76	+10.20	2.0	UTRAO	— UTRAO
			20‡	18.92 ± 0.15	1.94 ± 0.10	+0.26 ± 0.20						

Aperture diameter 15" unless otherwise specified.

* On February 16, 20 and 22, 1974 the 1P21 system was used, and on February 19, 1974 the RCA 4516 system.

† The following corrections have been applied for galactic extinction¹⁷: In *V*, -0.18 cosec *b*^{II}; in *B-V*, -0.06 cosec *b*^{II}, and in *U-B*, -0.0432 *b*^{II}.

‡ A 7" diameter aperture was used

§ A 29" diameter aperture was used

¶ RSO, NSO: Red or neutral stellar object.

|| Texas, unpublished.

lie even further below the main sequence and to the right, in the *U-B*, *B-V* diagram. Objects of higher redshift will have an even greater proportion of their light output in the *V* filter. A useful criterion for the selection of candidates for QSOs having $z > 3$ may be broadband colours extending to longer effective wavelength, say *U*, *V*, *R* and *I*, or by comparison of images on ultraviolet, red and infrared plates, analogous to the

technique used by Braccetti *et al.*¹⁸ to discover lower redshift QSOs.

Two of the objects observed (3C66A and 1147+245 (OM 280)) lie in the region of the class *c* objects, above. The results for 3C66A have already been discussed¹⁴. No lines have been found yet in the optical spectrum of 1147+245 (refs 15, 16).

Among the red stellar objects, the candidate identification for 1430+095 (OQ050) lies close to the main-sequence curve in Fig. 1 and a spectrogram later confirmed that the object is a late type star and therefore probably not associated with the radio source. Another red object, within 3" of 0850-034 (4C-04.17), lies fairly near the main sequence curve, in the region also occupied by radio galaxies. Only weak spectra of this object have been obtained by Derek Wills and me; there are certainly no strong emission lines, but the possibility of stellar absorption lines is not excluded. The remaining red object, associated with 1600+148 (4C14.61), should probably be classified as a galaxy on the basis of the present photometry. Image-tube photographs (scale 25" mm⁻¹) confirm the stellar nature of the image seen on the PSS; again, however, only weak spectrograms have been obtained ($B \approx 20.5$) sufficient only to rule out the presence of very strong emission lines. The remaining stellar object in Table 1, 1049+616 (4C61.20), has a redshift¹⁶ $z = 0.422$. Wills and Wills¹⁶ remarked that the spectrum was somewhat more like that of a galaxy than of a QSO, but the present results show that its colours are quite typical of those of the majority of QSOs.

Two galaxies were also observed; they each appear compact (but definitely non-stellar) in the PSS. Of these, 0456-043 is the more compact and shows emission lines in the spectrum ($z \approx 0.118$); the other, 1037+302 (4C30.19), shows no emission lines. The colours of these two objects are quite similar.

The present photometry has been valuable in attempting to classify objects on the basis of their continuum colours, especially for the apparently stellar candidate radio source identifications where no lines have been seen in the optical spectrum. The colours of the high redshift QSOs, OH471 and OQ172, lead me to suggest that broadband photographic or wide photographic photometry at a few effective wavelengths over a wide wavelength range (extending into the infrared) may be a practical alternative to using the properties of radio emission and good radio-optical position agreement in selecting high redshift ($z \geq 3$) QSOs; it may therefore be a useful way to test whether or not radio-emitting QSOs are a representative sample of QSOs as a whole.

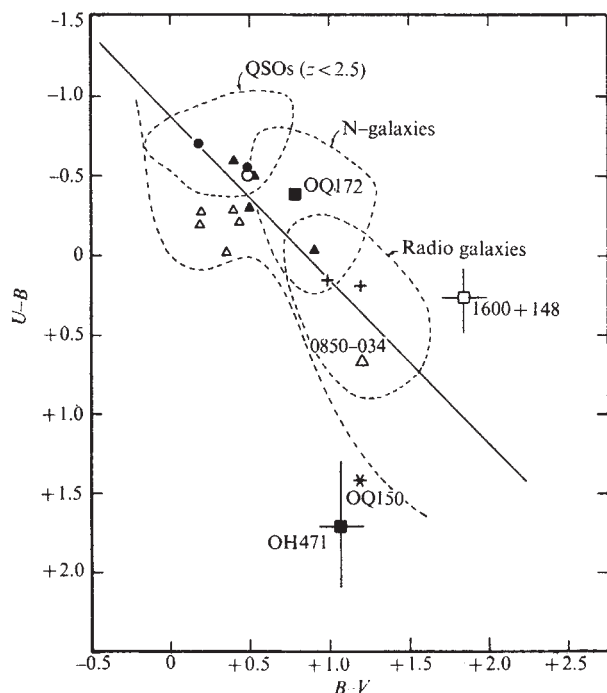


Fig. 1 New colours for some radio source identifications compared with typical colours for QSOs ($z < 2.5$), N-type galaxies, OJ287-type objects and radio galaxies. Error bars shown for OH471 and 1600+148 indicate the dominant contributions from counting statistics. The average main sequence and blackbody curves are shown. Data for the QSOs PHL957 and 4C05.34 have been taken from Sandage¹⁸, and unpublished data for three other QSOs having $z \sim 2.5$ have been supplied by R. Lynds and D. Wills. For OJ287-like objects, data are from ref. 19. The symbols refer to colours for new QSOs (●), OJ287-type objects (▲), QSOs having redshifts $2.5 < z < 3.0$ (△), galaxies (+), and 3C66A (○).

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observed during a time interval of more than an hour) more than 300 spectrograms were obtained.

The spectrograms and registrograms show the complicated character of the spectrum with emission (as well as absorption) features. Some successive registrograms of spectra of H_α and H_β regions obtained with a one-minute interval and spectra obtained on different nights are shown in Figs 1 and 2 (the field size of the spectrograph camera does not permit one to obtain H_α and H_β lines on the same spectrogram). The differences in spectra are easily seen. Many details are repeated, but sometimes (for instance on August 26) the emission features in the region of H_α are absent (Fig. 2). Some absorption features of this spectrum correspond to emissions in the spectra of August 28 and September 7. Inspection of the spectra shows that there are continuous intensity variations of many emission features and absorption lines (and probably of the H_α and H_β lines) with the shortest time scale of the order of several minutes.

The complicated character of the spectrum, its variability and low resolution do not permit one to identify emission details with certainty as yet.

Approximate wavelengths of the strongest emissions (determined with an accuracy of 2–3 Å) correspond to FeII, VII, CrII, HfII, TiII, ArII, CIII as frequently as to GdII, LaII, CeII, NdII, SmII. (According to Preston⁸, lines of neutral atoms in the spectra, apart from H and HeI, are probably

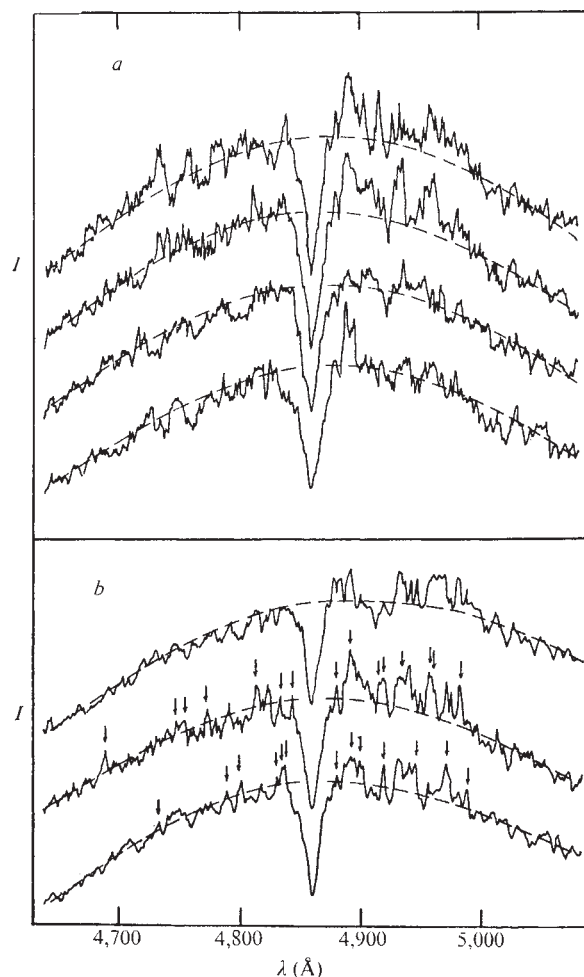


Fig. 1 *a*, Example of registrograms of successive spectra of HD215441, obtained on September 7, 1973, with a one-minute interval in the region of H_β . *b* Registrograms of spectra, obtained on different nights. Dashed curves show conventional continuum. Top, August 26, 1973; centre, August 28, 1973; bottom September 7, 1973.

Rapid variations in the spectrum of the magnetic variable star HD215441

THE star HD215441 has the largest magnetic field ever recorded, about 34 Kgauss (ref. 1), varying without change of polarity. There are some indications of smooth brightness variations of this star with amplitude $\Delta m \approx 0.2$ mag as well as of small smooth variations of linear polarisation correlated with the brightness variation²⁻⁴.

Rapid fluctuations (during several minutes) of brightness (W. Schöreich and R. Panov, unpublished) and polarisation in a wide spectral interval as well as polarisation in the wing of the H_β line⁶, were also observed. Photoelectric measurements with narrow and wide passband filters showed variations of equivalent width of H_β by 30% within 90 s. Similar variations have not been detected for other Balmer lines⁷.

Some efforts to detect rapid spectral variations of the star HD215441 are described below.

Our observations were carried out with a spectrograph and three-stage image tube (with a multi-alkali photocathode) at the Nasmyth focus of the 2.6 m telescope of the Crimean observatory. The dispersion was about 37 Å mm^{-1} at H_α and 46 Å mm^{-1} at H_β , the resolution being about 3 Å. In favourable observing conditions exposure times were 60 s for H_α and 25 s for H_β . During observations on August 25, 26, 28, and September 7, 1973, and January 2, 1974 (when the star was

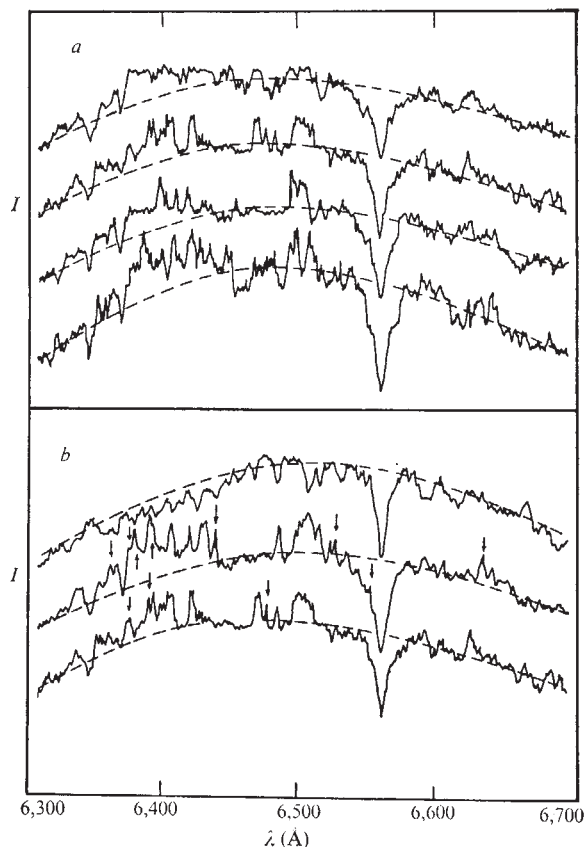


Fig. 2 As Fig. 1 but for H_{α} .

absent.) Positions of some rare earth lines are denoted by arrows on Figs 1b and 2b.

The appearance of rare earth lines in the spectrum of HD215441 is not improbable. Extremely variable emission features point to rapid non-stationary processes on the surface of the star, and it is not ruled out that these processes are similar to solar flares. In flares the lines of rare earths are sometimes very prominent^{9,10}. On the other hand, an abnormally high abundance of rare earths is a peculiar feature of some magnetic stars.

As some emission details can appear in the wings of hydrogen lines their equivalent widths can change due to blending. Thus the equivalent width variations of the H_{β} line found by Wood⁷ might be, more or less, caused by this effect or by a variable emission close to the line.

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Photometry of the zodiacal light in the near infrared

DURING our investigation¹ of comet Kohoutek (1973f) we had occasion to take many photographs of the zodiacal light through various filters. In particular we photographed it in infrared light with filters to isolate regions from 610 to 920 nm. Later we calibrated the emulsions and camera lenses together by photographing suitably dilute sunlight scattered by a fresh MgO surface.

After making allowance for airglow emission it was clear that the albedo of the zodiacal dust cloud is greater, by a factor of two or more, in the region 750 to 920 nm than in the visible. This is in contrast to the results of Matsumoto². Attempts to observe a reddening of the visible light have been rather inconclusive³.

Our result is interesting for several reasons. We might expect reddening for a very narrow size range of scatters with radii of about 10^{-6} m (ref. 4) but this would be disguised if the usually assumed size distributions are correct.

The work of Johnson and Fanale⁵ on crushed meteorite fragments provides some evidence for a high infrared albedo for asteroidal material (especially for particles of radius $<10^{-4}$ m) but not to the extent that the present observations showed. It is difficult to account for this infrared excess without introducing further hypotheses.

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High concentrations of ice particles in a layer cloud

CONCENTRATIONS of ice particles in clouds, greatly in excess of those expected from ice nucleus measurements have been reported^{1,2}. Most of these observations have been made, however, in cumulus or other complex cloud systems, which increases difficulties in interpretation. Here I describe measurements of high concentrations of ice crystals in a simple, non-convective cloud layer.

The measurements were made in very light snowfall (which never exceeded a 'trace' in equivalent precipitation rate) at Snoqualmie Pass, Washington State, between 1045h and 1300h LT on November 28, 1972. The distribution of cloud, winds and temperatures measured with a rawinsonde, and the locations at which airborne and ground measurements were made is shown in Fig. 1. The cloud top, as observed from the aircraft, was very smooth and uniform and located at 1,830 m (all altitudes are above mean sealevel) where the temperature was -5° C. Cloud base was at 1,220 m, where the temperature was

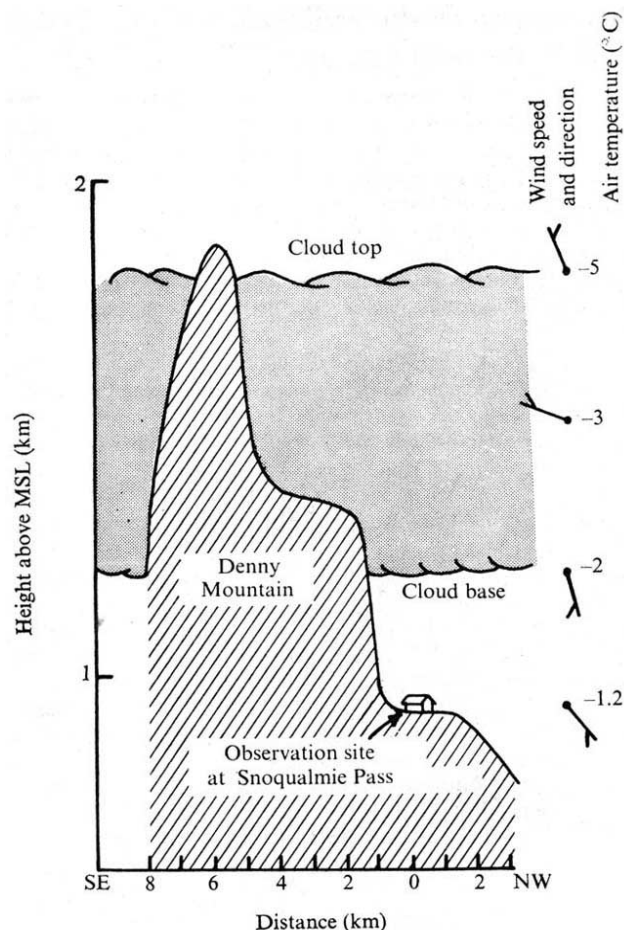


Fig. 1 Observation site and atmospheric conditions.

-2°C . A spectrum of cloud droplet sizes was obtained near the cloud top with a Formvar cloud particle sampler attached to the aircraft (Fig. 2). Also shown in this diagram is the contribution to the liquid water content from droplets of various sizes; droplets with diameters up to $75\text{ }\mu\text{m}$ were present.

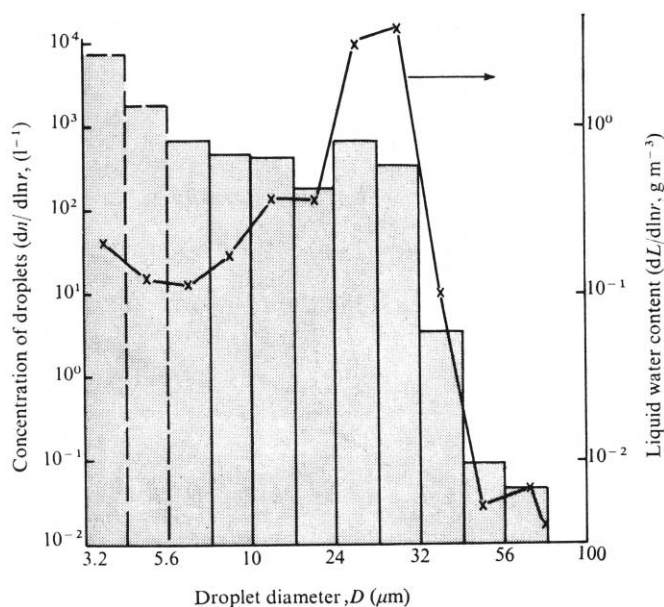


Fig. 2 Size distribution of cloud droplets (shaded) and liquid water content near the top of the cloud. The total liquid water content was 1.1 g m^{-3} , and the total concentration of droplets greater than $5\text{ }\mu\text{m}$ diameter was 360 cm^{-3} . Droplet concentrations below about $5\text{ }\mu\text{m}$ are unreliable because of uncertainties of collection.

Some isolated ice needles and irregular crystals were also collected from the aircraft flying near the cloud top.

The crystals reaching the ground site (elevation 915 m) initially consisted of single needles and sheaths, but as the snowfall rate increased they formed aggregates. As the number of crystals in an aggregate is difficult to estimate, the results described here are confined to the period when single crystals only were collected. The concentrations of single crystals in the air were deduced from measurements of the number of crystals which fell on to glass slides exposed horizontally for measured time intervals, by considering the speeds of fall of needles and sheaths ($\approx 1\text{ m s}^{-1}$)³. The concentrations of crystals in the air was about 1 l^{-1} . Measurements of atmospheric ice nuclei indicated that at the minimum temperature in the cloud (-5°C), the concentration of ice nuclei should be about 10^{-4} to 10^{-3} l^{-1} . Therefore, the concentrations of the crystals in this simple layer cloud exceeded, by at least a factor of 10^3 , those expected from measurements of ice nuclei.

The size distributions of the crystals collected on the ground are shown in Fig. 3. The crystals tended to be either completely unrimed or moderately to densely rimed. The droplets frozen on to rimed crystals were up to $100\text{ }\mu\text{m}$ in diameter and were concentrated on the leading edges of the crystals. The rimed

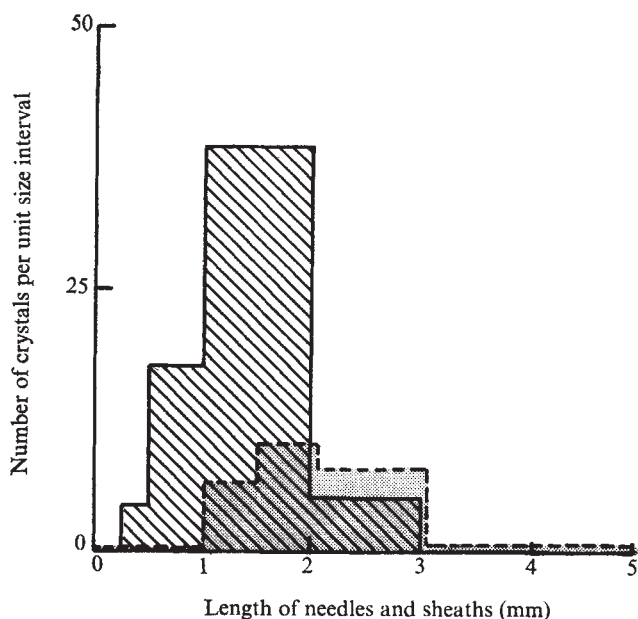


Fig. 3 Size distribution of rimed (shaded) and unrimed (hatched) needles and sheaths collected at the observation site.

crystals tended to be fewer in number but larger in size than the unrimed crystals, indicating that the unrimed crystals had shorter growth times. The unrimed crystals possibly originated on ice splinters produced during riming in the lower regions of the cloud. In this connection, we note that the conditions under which riming occurred in this cloud encompass the critical combination of conditions for temperature, droplet sizes and impact speeds under which large numbers of splinters have been observed in laboratory experiments⁴. As unusually high concentrations of ice crystals, however, have been observed in clouds where there was no riming (G. Vali, personal communication) and, in some situations, do not occur in clouds where riming does take place⁵, there is still no completely satisfactory theory to explain the phenomenon.

The high concentrations of ice crystals which occurred in the cloud described here could not have been produced by the mechanism postulated by Mason⁶ because that requires convective towers. Indeed, contrary to Mason's contention, there are several sets of independent field observations which

show high concentrations of ice crystals in non-convective clouds (refs 2 and 7, and G. Vali, personal communication).

Because there are natural limits to the masses and speeds of fall which ice crystals can achieve in the atmosphere, ice crystal concentrations of the order of 1 l^{-1} are needed to produce even light snowfalls. Such concentrations must, therefore, be quite common in shallow layer clouds. For example, a needle crystal of $5 \mu\text{g}$ mass has a speed of fall of about 1 m s^{-1} . To produce an equivalent water precipitation rate as small as 0.02 mm h^{-1} , these crystals have to exist in the air in concentrations of about 1 l^{-1} .

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Mercury in the sediments of the Pallanza Basin

SURFACE sediment samples (0–3 cm) taken from the Pallanza Basin of Lago Maggiore, Italy, in 1970 have been analysed for mercury, using flameless atomic absorption spectrophotometry. The concentration of mercury in the Maggiore sediments proved to be extremely high when compared with other reported lake sediments (Table 1, and ref. 1). They were checked by another laboratory and both sets of results were in extremely good agreement. Mercury concentrations ranged from 0.2 to 20.1 p.p.m., with a mean of 7.7 p.p.m. and a standard deviation of 4.9 p.p.m.

The highest concentrations of mercury in the Pallanza Basin occur to the south of Pallanza, with additional elevated regions occurring in the nearshore area to the north of Stresa and off Feriolo (Fig. 1a). Thomas² has demonstrated the use of a quartz correction for total mercury in assessing the variable adsorption of mercury by sediment fines and in locating point source

inputs. A similar correction has been applied for the Pallanza Basin sediments, in which total observed mercury has been corrected for the percentage of material of sand size (Fig. 1b). Mercury enriched sediment occurs predominantly along the north shore of the basin, in water which is up to approximately 125 m deep, with the Toce River as the indicated primary source. The areas of higher concentration at Feriolo and Stresa are again apparent from the distribution given in Fig. 1b.

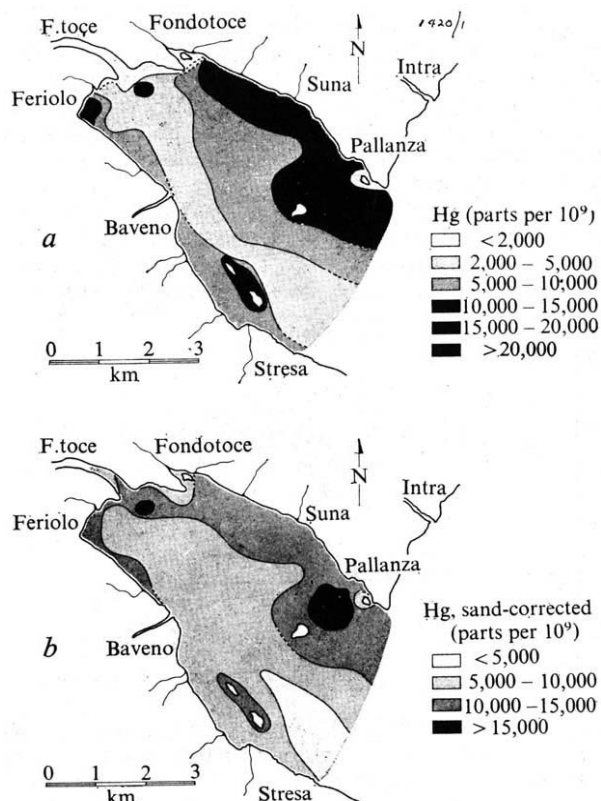


Fig. 1 a, Distribution of total observed mercury in the surface sediments of the Pallanza Basin; b, distribution of sand corrected mercury in the sediments of the Pallanza Basin.

After the start of summer stratification, in late March, suspended materials associated with Toce River waters occur predominantly to a maximum depth of 50 m in the Pallanza Basin^{3,4}. Thus, through the bulk of the basin, turbid Toce River water flows over the hypolimnion and is transported to the open waters of Lago Maggiore. In the shallow water areas, suspended solids, associated predominantly with the epilimnion, are deposited and retained. The maximum input of solids from the Toce River takes place with the spring runoff which occurs in late April and May, postdating the start of lake stratification⁴.

Table 1 Mercury concentrations in some lacustrine, surface sediments*

Lake	No. of samples	Depth of sample (cm)	Concentration (parts per 10 ⁹)			
			Minimum	Maximum	Mean	Standard deviation
Superior	405	0–3	4	584	83	56
South Michigan	31	Variable	30	380	146	
North Channel	55	0–3	8	1,112	151	231
Huron Georgian Bay	117	0–3	12	9,500	257	1,013
Huron	163	0–3	54	805	222	162
St Clair	55	0–2	70	2,565	632	628
Erie	243	0–3	13	7,488	609	703
Ontario	248	0–3	32	2,100	651	507
Pallanza Basin, Lago Maggiore	34	0–3	240	20,100	7,649	4,882

* Data from ref. 1.

The relationship of total mercury to other sedimentary parameters has been examined by means of a linear correlation matrix. Significant relationships were observed with the clay size fraction ($r = 0.722$) and with iron ($r = 0.553$), suggesting that the mercury is associated with iron rich, detrital clay particles (see ref. 5). No significant relationship with organic carbon ($r = 0.367$) was observed.

The impact of the high mercury concentrations on the aquatic biota, has not been examined. Damiani⁶ discussed the variation of the C/N ratio in the sediments of the basin and noted that lower ratios, attributable to autochthonous organic matter, occurred in the shallow water areas along the north shore from Fondotoce to Pallanza, north of Stresa and off Feriolo. Higher C/N ratios, indicative of allochthonous organic matter, were observed in the deeper central waters of the Pallanza Basin and were attributed to direct derivation from the Toce River. Figure 2 shows the relationship of mercury to the C/N ratio; the relationship is significant, with a correlation coefficient of -0.656 . Even though the gross statistical relationships show that the mercury is associated predominantly with the clays, the relationship of mercury with the C/N ratio implies that the mercury may in part, be associated with autochthonous organic

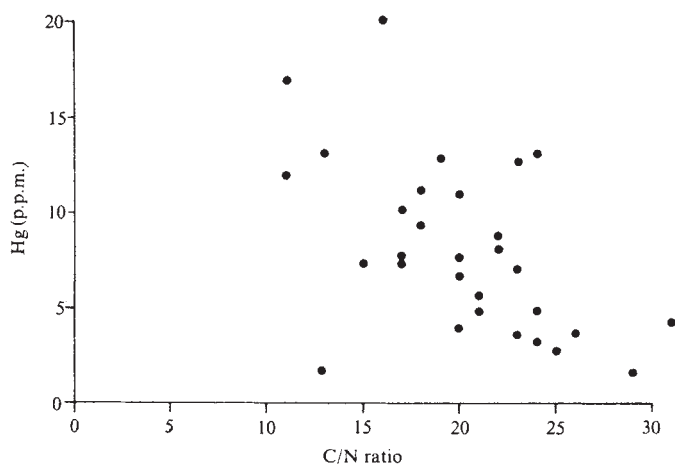


Fig. 2 Relationship of mercury to the C/N ratio in the sediments of the Pallanza Basin.

matter. This is in distinct contrast to the lower mercury levels in allochthonous organic matter with a high C/N ratio, derived from the Toce River. It is possible that this relationship is coincidental in that the deposition of clay and associated mercury from the Toce River occurs predominantly in the shallow water regions which in turn receive the highest input of deposited autochthonous organic matter. We feel, however, that the degree of the relationship between mercury and autochthonous organic matter, as inferred from the C/N ratio, implies uptake of mercury by the primary producers in the shallow water zones, which indicates that mercury transfer processes are taking place. Confirmation may follow from an examination of the levels of mercury concentration in the biota of the lake.

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Drag reduction in polymer mixtures and the effect of molecular weight distribution

SMALL amounts of certain high molecular weight polymers can reduce the frictional drag in turbulent flow through pipes^{1,2}. Although the phenomenon—the so-called drag reduction effect—has been studied extensively, very little information is available about the effects of molecular weight distribution in polydisperse samples. All common drag reducing agents are characterised by a broad distribution of molecular weights, which changes during shear degradation^{3,4}. Consequently, any molecular theory of drag reduction must include some means of handling these complications. The equations presented here represent a possible first step in this direction.

For many materials the percentage of frictional drag reduction at a fixed Reynolds number, DR , can be related to a reduced polymer concentration, r , by the empirical equation^{5,6}

$$DR/DR_{\max} = r/(1+r) \quad (1)$$

where

$$r = c/[c] \quad (2)$$

The polymer concentration, in weight parts per million (w.p.p.m.), is represented by c , and $[c]$ and $DR_{\max}$ are empirical constants called the intrinsic concentration and the maximum drag reduction, respectively. These equations are valid at flow rates significantly above the onset of drag reduction and at relatively low concentrations, where DR is substantially less than $DR_{\max}$. At higher concentrations the measured drag reduction is often less than that predicted by equation (1) and thus $DR_{\max}$ is seldom obtained in experiments. This is parti-

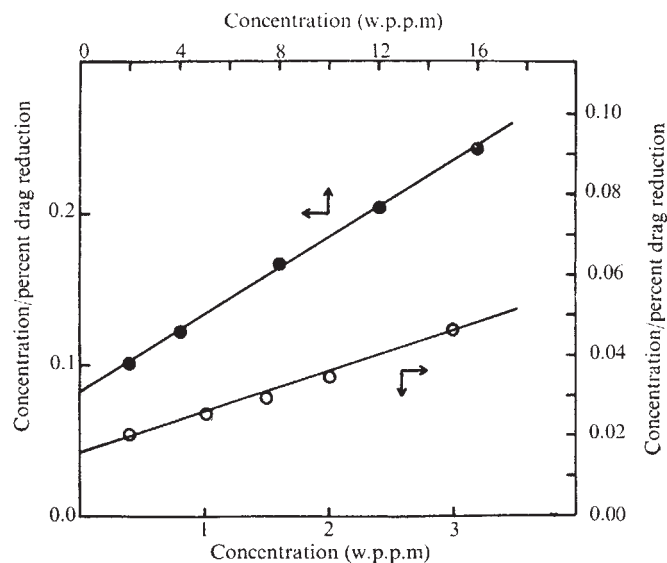


Fig. 1 Graph of concentration over percent drag reduction against concentration: ●, WSR-205 data; ○, WSR-301 data. Slope equals $(1/DR_{\max})$; intercept on the ordinate equals $([c]/DR_{\max})$.

Table 1 Properties of polymer samples

Compound	[c]	DR _{max}
WSR-205	8.0	98
WSR-301	1.5	98

cularly noticeable in capillary tube measurements where $DR_{\max}$ can be quite large. The intrinsic concentration is a measure of the polymer's effectiveness as a drag reducing additive; that is, materials with the lowest values for [c] are the most effective.

Little, Ting and Kim have studied the drag reducing ability of polyethylene oxide⁶ and polyacrylamide (unpublished work). They found that equations (1) and (2) are obeyed at low concentrations and that [c] can be related to the weight average molecular weight, $\bar{M}$:

$$[c] = A/(\bar{M} - M_0) \quad (3)$$

where A is a constant characteristic of the polymer family and M_0 is the molecular weight below which no drag reduction is observed in that family. The weight average molecular weight can be defined as

$$\bar{M} = \sum_i c_i M_i / c \quad (4)$$

where c_i is the concentration (w.p.p.m.) of molecules with molecular weight M_i . Equations (1) and (2) may now be written as

$$\frac{DR}{DR_{\max}} = \frac{\sum_i r_i}{(1 + \sum_i r_i)}$$

where r_i is the reduced concentration of the i th component

$$r_i = c_i / [c_i] \quad (6)$$

and $[c_i]$ is the intrinsic concentration of the i th component

$$[c_i] = \frac{A}{(M_i - M_0)} \quad \text{when } M_i > M_0 \quad (7)$$

$$[c_i] \rightarrow \infty \quad \text{when } M_i \leq M_0$$

Experimental results⁶ also indicate that $DR_{\max}$ varies with molecular weight but only for molecules where M_i is close to M_0 . If M is significantly greater than M_0 , the effects of these lower molecular weight molecules is small and so $DR_{\max}$ can be treated as a constant.

These results suggest that reduced concentrations should be

additive when the sample is considered to be a mixture of many different components, each with a single molecular weight. The best way to test these equations would be to study mixtures of several different monodisperse polymers from the same polymer family. Unfortunately, such samples are very difficult to obtain. An alternate but less satisfactory procedure is to test mixtures of polydisperse samples. Expressions for drag reduction with mixtures of polydisperse samples from the same polymer family can also be derived using equations (2-4). The results are similar to equations (6) and (7) except that M_i is replaced by $\bar{M}_i$, the weight average molecular weight of the i th component. This means that the reduced concentrations should be additive for mixtures of polydisperse samples as well as for mixtures of monodisperse samples.

To test this, two high molecular weight polyethylene oxide samples were obtained (Union Carbide Corp.). An automated capillary rheometer⁷ was used to measure the concentration dependence of drag reduction for aqueous solutions of both samples at a Reynolds number of 9,000. The capillary tube has an inside diameter of 0.1575 cm. The intrinsic concentrations and maximum drag reductions were then determined⁶ from the slopes and intercepts in graphical plots of c/DR against c . Figure 1 shows graphs for the two samples, and in both cases the linearity is excellent. The values of [c] and $DR_{\max}$ are listed in Table 1. Various mixtures of these two samples were then prepared and their drag reduction measured at a Reynolds number of 9,000. The results are listed in Table 2.

In each case the predicted and experimental results are in agreement in spite of the fact that these mixtures represent a variety of distinctly different average molecular weights and molecular weight distributions. This suggests that equations (5-7) may represent a meaningful first step towards understanding the behaviour of mixtures and, therefore, the effects of molecular weight distribution. It is hoped that, as monodisperse drag reducing polymers become available, these relationships can be more rigorously tested.

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Role of upper-layer interactions in electron diffraction symmetries

STEEDS *et al.* have published pictures showing effects of upper-layer interactions in molybdenite^{1,2}. Because of its use in structure analysis it seems worthwhile to set down rules from the literature which relate electron diffraction symmetries to three-dimensional crystal symmetries. As distinct from problems which can be solved by considering only zero-layer interactions, such as the determination of a polar axis in the plane of projection^{3,4}, the total crystal symmetry, including the boundaries, must be considered when upper-layer interactions are important. As a simple example some results obtained from a highly tilted crystal are given, and I also comment on the conclusions of Steeds *et al.*^{1,2}.

There are two rules pertaining to the symmetry of the zone-

Table 2 Measured and predicted* drag reduction in mixtures

WSR-205 concentra- tion (w.p.p.m.)	Drag reduction at indicated concentration (w.p.p.m.) of WSR-301						
	0	0.25	0.5	1.0	1.5	2	3
0			24(25)	40(39)	52(49)	59(56)	65(65)
0.5		19(18)	27(26)	41(41)	51(51)		
1.0		22(22)	30(31)	43(43)	54(52)		
2.0	20(20)	29(29)	34(36)	47(47)	55(54)		
4.0	33(33)	36(39)	44(45)	53(53)			
8.0	48(49)	52(53)	57(56)				
12.0	59(59)						
16.0	66(65)						

*Values predicted from equations (6) and (7) are in parentheses.

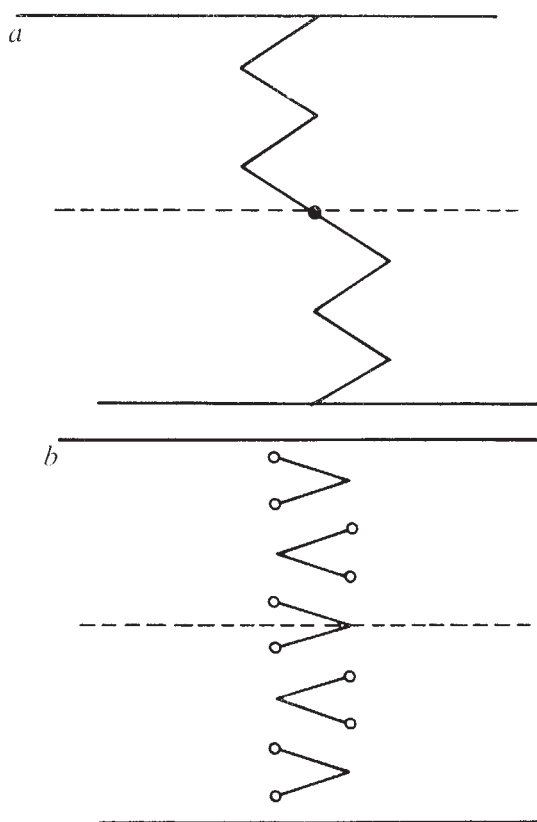


Fig. 1 The significance of a central mirror plane to the zero-beam diffraction symmetry. *a*, Representation of a crystal with the stacking sequence A-B-A-B-A-C-A-C-A. This illustrates the case of a graphite crystal with a central stacking fault. This crystal has no central mirror plane or glide plane, although it has a centre of symmetry¹². The zero-beam distribution resulting from diffraction will be non-centrosymmetric. *b*, Representation of a molybdenite crystal of five layers ($2\frac{1}{2}$ unit cells), which has a central mirror plane but not a centre of symmetry. The zero-beam distribution will be centrosymmetric. Empirical results are dependent on structure (Table 1). Ray diagrams, incorporating the law of reciprocity, given in publications referenced in the text, make these conclusions obvious.

axis pattern^{3,5,6}. First, the condition for a centre of symmetry in the zero-beam pattern is that the crystal as a whole has a mirror or glide plane, perpendicular to the zone axis, midway between its surfaces. Second, an additional condition for a centre of symmetry in the remainder of the diffraction pattern is that there is a centre of symmetry in the two-dimensional projection of the crystal.

These conditions ((1) and (2)) relate, respectively, to upper-layer and zero-layer interactions. The latter terms need definitions appropriate to dynamic scattering. In discussions of X-ray crystallography 'upper layer' and 'zero-layer' are used for a particular zone independent of the scattering experiment, and/or an infinite crystal (thus, crystal boundaries are irrelevant to this nomenclature). It is much better to use another nomenclature for electron diffraction.

'Zero-layer reflections' are defined here as those reflections generated by scattering from the z -independent structure, that is, from the finite crystal with a structure averaged out in the z -direction between the crystal surfaces, where the z -direction is identified with the surface normal. This definition, following

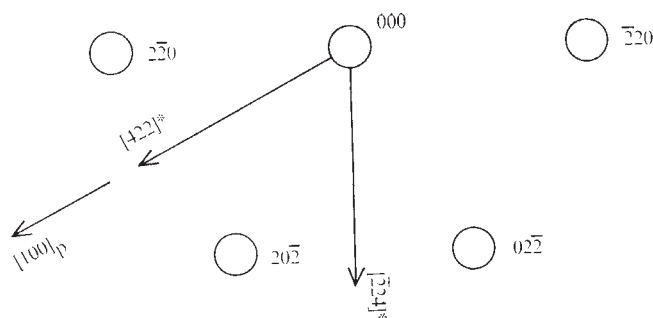


Fig. 2 Indexing of reflections relative to the projection of the surface normal, $[100] p$, for the $[111]$ pattern; $02\bar{2}$, $02\bar{2}$, are zero-layer reflections. Other 220-indexed reflections, using the present nomenclature, belong to first upper and lower layers.

the scattering process rather than the unit-cell geometry, allows 'upper-layer interactions' to be associated with effects strictly connected with propagation. This is consistent with established theory⁷⁻⁹.

Condition (1) can only be fulfilled for a parallel-plate crystal when the surface normal is parallel to the zone axis. This condition refers to the symmetry of the crystal as a whole rather than simply to the space-group symmetries of the unit cell. Condition (1), and thus the zero-beam symmetry, has no direct bearing on the question of whether the crystal as a whole, or the unit cell, has a centre of symmetry (Figs 1*a*, *b*, and 2). Both conditions taken together determine a centrosymmetric crystal. The two conditions can, however, be exploited separately in analyses (Table 1).

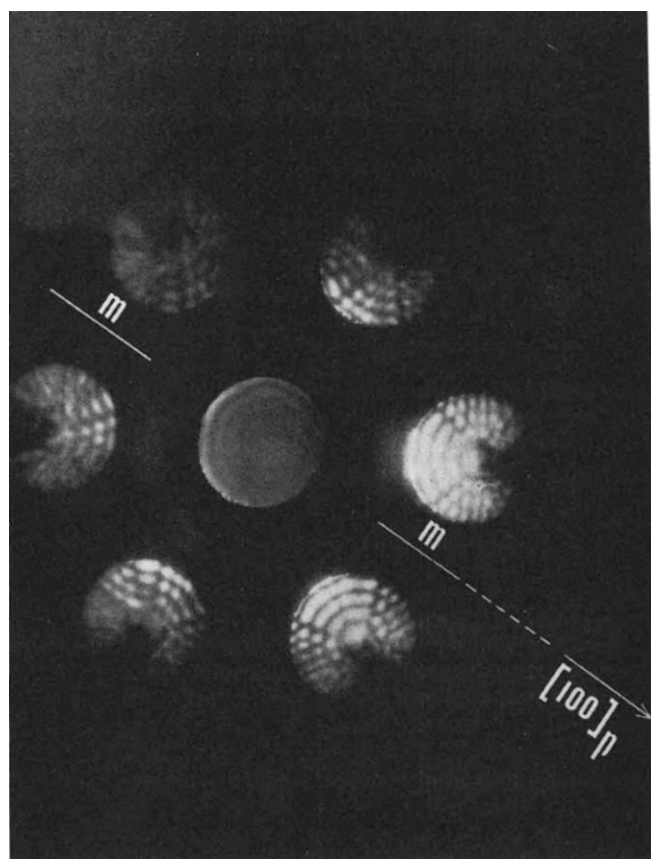


Fig. 3 Convergent-beam diffraction pattern from an MgO platelet with (100) faces, at the $[111]$ zone axis, using 80 kV electrons. Direction of the projection of $[100]$ is indicated. The only symmetry in the diffraction pattern including the zero beam is a mirror line perpendicular to this direction.

Table 1 Effect of structure on empirical results

	Crystal symmetry	Diffraction symmetry	
		Zero beam	Rest of pattern
(1) Centrosymmetric crystal	Central mirror plane No central mirror plane (examples: Figs 1a and 2)	Centrosymmetric Non-centrosymmetric	Centrosymmetric Non-centrosymmetric
(2) Non-centrosymmetric crystal, centrosymmetric in projection	No central mirror plane	Detectably non-centrosymmetric only for a large (polar) c -axis, or long disorder parameter in the c direction	
(3) Crystal non-centrosymmetric in projection	Central mirror plane (example: CdS, BaTiO ₃ , space group symmetry, Fig. 1b (crystal boundaries))	Centrosymmetric	Non-centrosymmetric
	No central mirror plane	Detectably non-centrosymmetric as for (2)	Non-centrosymmetric

As an example in which upper-layer interactions strongly influence the symmetry of the diffraction pattern, consider the [111] zone-axis pattern obtained from an MgO plate with (100) surfaces. I reason that the hexagonal symmetry of a crystal with [111] surfaces will be destroyed by upper-layer interactions. The pattern will only have one mirror line, through its centre, parallel to the set of mirror planes of the whole crystal, that is, parallel to planes containing [100] and [111] (Fig. 3). The pattern symmetry corresponds directly to the three-dimensional symmetry of the crystal in that orientation.

The effect of symmetry on the zero-beam can be increased by tilting the crystal away from the axis, which increases the dynamic coupling. Figure 4 shows the convergent-beam picture for the 'three-beam pattern' near $\zeta_{02\bar{2}} = 0$ and $\zeta_{20\bar{2}} = 0$. With

this indexing the projection of the surface normal, [100], is parallel to $[42\bar{2}]^*$. As the space-group mirror line $[22\bar{4}]^*$, bisecting the two scattering vectors, is not contained in a mirror plane of the whole crystal, the two reflections have unequal intensity distributions. The asymmetry in the zero-beam

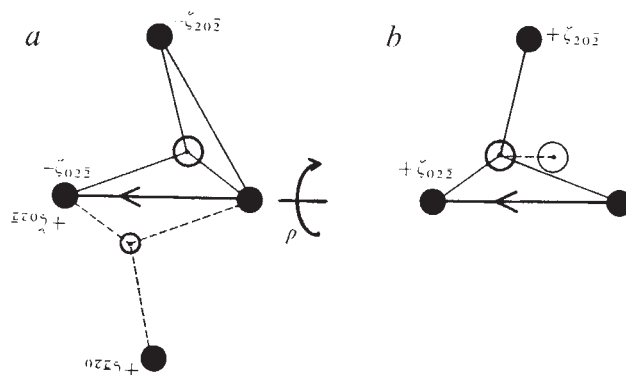


Fig. 5 *a*, The reciprocity construction of Moodie⁴ projected on to the plane perpendicular to [111]. The centre of the Ewald sphere is shown encircled; arrow, scattering vector for 022. The reciprocally related diagram, below the plane of projection, is indicated by broken lines, and its excitation errors and their signs are shown inverted. *b*, Result of rotating the reciprocal diagram of *a* through 180° about the scattering vector. The original Ewald sphere centre is also shown, indicating that its displacement is parallel to the diffraction vector.

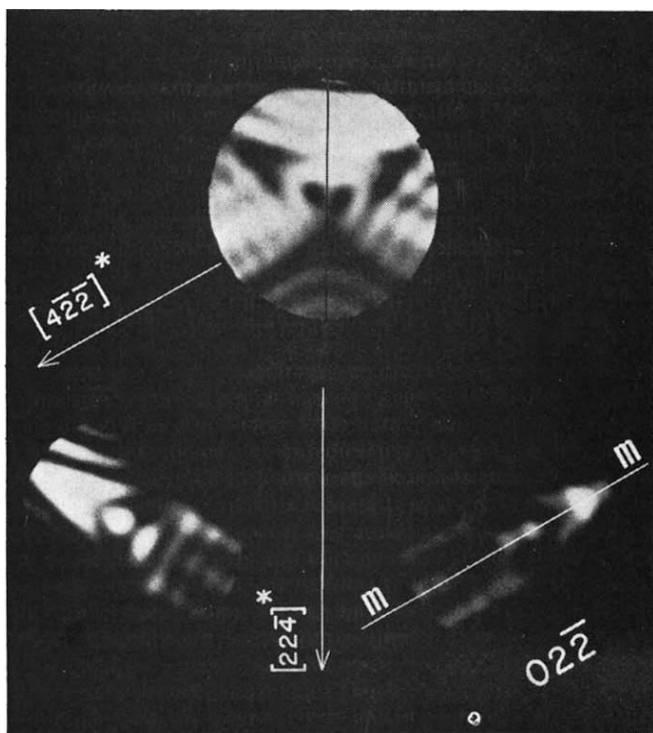


Fig. 4 Convergent-beam diffraction from the crystal used for Fig. 3 tilted to satisfy the 022 and 202 reflections. With this indexing the projected [100] surface normal is parallel to $[42\bar{2}]^*$, which is also parallel to the mirror planes of the crystal. The 022 reflection is symmetric about the line $m-m$ of zero excitation error.

distribution about this line demonstrates the upper-layer origin of this effect. An almost perfect mirror line is, however, observed through the 022 distribution along the line $\zeta_{02\bar{2}} = 0$. This line is parallel to the mirror plane of the crystal.

It is important to ascertain whether symmetries apparent in the pattern are expected to be exact, or only approximate, theoretically, because the former case provides a good test of experimental accuracy. The construction given by Moodie⁶ for the application of the reciprocity theorem allows determination of the symmetry of the 022 reflection for this crystal. This construction gives the two incident directions leading to identical scattering vectors and amplitudes for any crystal (Fig. 5a). Equivalent incident directions are obtained by operations on the diagrams which, because of symmetries, leave the crystal unchanged. To exclude the trivial exercise of equating intensities from crystallographically equivalent zone axes, these operations must leave both crystal and zone axis unchanged.

Equivalent conditions can be found for the 022 beam by rotating the diagram through 180° (Fig. 5b). This operation will leave the crystal unchanged provided it is centrosymmetric (that is, if conditions (1) and (2) are fulfilled) (Fig. 6). Figure 5 gives proof of the existence of a mirror line in the 022 distribu-

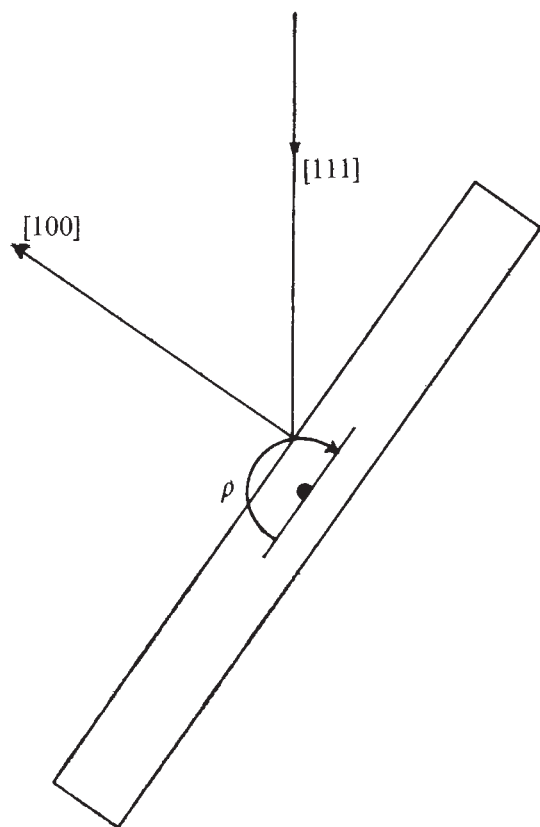


Fig. 6 Diagram showing the tilted crystal. Relationship between surface normal [100], zone axis [111], and the 180° rotation ρ about the 022 scattering vector are shown.

tion, for the centrosymmetric MgO structure. The setting of Fig. 4 thus provides the additional information necessary for interpretation of Fig. 3: the asymmetry at the zone axis arises from the influence of crystal boundaries and not from a polar structure. This conclusion follows directly from the proof of Fig. 5.

There are published observations of the effects on symmetry of stacking faults¹⁰. In MoS₂ the most common cause will be a S/S shear¹¹ somewhere central in the crystal. The problem is closely related to that in graphite, for which complete calculations exist¹². In the case of a pure rhombohedral crystal there is a regular rather than an irregular sequence of basal faults, over a finite thickness. I believe that distinguishing these cases from the data, and locating the positions of faults, requires numerical analysis, and that the rules quoted by Steeds *et al.*² are not always followed. As extreme examples I refer to the upper-layer unit cell (regular stacking) effects in gold¹³. In these cases there are antisymmetric contours over relatively large angles away from the [111] zone. There is also a strong trigonal pattern caused by a single stacking fault in graphite¹².

Diffraction contoured micrographs allow exploration of a large angular region using one diffraction order, but lack linear mapping and fine specimen resolution, as crystal curvature cannot be controlled. On the other hand, convergent-beam diffraction allows specimen resolution down to 50 Å, with accurate angular resolution, but over a necessarily limited angular region. Wide-angle convergent-beam patterns¹⁴ (with overlapping diffraction orders) can be shown to have arbitrarily imposed symmetries, although a series of normal Kossel-Möllenstedt patterns (non-overlapping orders) can be used to map out the entire region of the zone axis. As specimen resolution is, however, limited, this is not a universal technique, and

normal electron micrographs are also required. The relative advantages of either of the conjugate objective planes for structure analysis will be discussed in future (W. C. T. Dowell, and P. Goodman, unpublished).

The wavelength dependence of patterns of the zone axis provides a simple test¹⁵ of the structure factor as distinct from shape-transform⁶ dependence. It is misleading, however, to refer to the two-beam approximation in connection with the low-voltage (or large excitation error) zone-axis pattern, and more correct (A. F. Moodie, unpublished) to refer to a different approximation (for example, the second Born approximation, or the thick phase-grating¹⁶ approximation).

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DR STEEDS REPLIES: Dr Goodman¹ casts doubt on the bright field rotation method² of identifying the presence of faults parallel to the surface of a specimen. The examples quoted in support of his point of view are, however, inadequate. Whereas our experiments are often performed over rotations of 20° or more, in the convergent beam work in gold, cited by Dr. Goodman, the angular range in view is 1.29° at 4.21° from [111], which is much too limited for a test. During large rotations the systematics-only pattern is interrupted by various non-systematic reflections, but there are several ranges of orientation in which non-systematics are not important and the asymmetric pattern returns to essentially the same form. The graphite example is irrelevant because the field of view is restricted to a narrow angular range close to the [0001] zone axis where the pattern is dominated by rapidly varying cross-grating contributions; our 'rule' refers to contours which result essentially from one-dimensional diffraction. Clearly, the misunderstanding arises from the very great difference of angular view in convergent beam and bend contour patterns, despite their formal equivalence (to a first approximation). Effects of inclination, although important for really large tilts, such as the 54° from the normal of the foil used by Dr Goodman, are not in our experience significant for smaller rotations of the order which we propose in our experimental test, at least not for bend contour patterns, where a certain amount of distortion is almost inevitable.

Dr Goodman claims that our reference to "two-beam-like behaviour" is misleading, and so we wish to state explicitly what was meant by that phrase. It is possible to show from the many-beam theory of electron diffraction that (B. Buxton, unpublished)

$$\frac{d\xi_{ij}}{d\beta} = \xi_{ij} \left\{ \frac{1}{\beta} + \beta \gamma^2 \left[1 - \frac{\gamma}{s_i - s_j} \frac{d(s_i - s_j)}{d\gamma} \right] \right\}$$

$$s_i - s_j = (\gamma^{(i)} - \gamma^{(j)})/4\pi k$$

where $\gamma^{(i)}$, $\gamma^{(j)}$ are the eigen values obtained by matrix diagonalisation (usual notation³); ξ_{ij} is the extinction distance corresponding to $(i-j)$ dispersion surface transitions; $\beta = v/c$; and $\gamma = (1 - \beta^2)^{-1/2}$.

According to strict two-beam theory $d(s_i - s_j)/d\gamma = (s_i - s_j)/\gamma$ and so our result holds. There are, however, a number of other situations, which we call "two-beam-like", in which the second term in the above expression vanishes or is negligibly small. These often occur when there are only two branches of the dispersion surface with excitations which vary rapidly with orientation (characteristic low voltage behaviour). Even in extreme cases of many-beam diffraction, such as in the case of molybdenite in our illustration, the pattern can be regarded as coming largely from transitions between two branches, although the branches involved change with orientation.

We find Dr Goodman's change of definition of zero and upper layer reflections unnecessary and inconvenient⁴, involving as it does the surface normal. In our experience, the relative orientation of a zone axis and the surface normal may stray by 20° or more without significantly affecting zone axis patterns. Finally, although Dr Goodman's two conditions are sufficient they are not necessary. To take an extreme case not covered by condition (1) we may obtain a centre of symmetry in the zone beam pattern by diffraction from two different monolayers having centres of symmetry colinear with the mean direction of the incident beam (we are grateful to Professor Frank for making this point).

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A new penetrating-radiation image converter

A NEW practical penetrating-radiation television camera tube, termed the Metallonicon, has been built. It operates at X-ray frequencies, and a discernible image is obtained with two mr. s^{-1} incident on the target, which is comprised of a heavy metal layer in intimate contact with a secondary electron enhancing storage layer.

Most imaging systems used in the application of isotopes form an image in a large area scintillation counter crystal, the light output of which is detected by photo-multiplier tubes for storage, subsequent processing and image formation¹. The work reported here has as its goal the combination of the above image forming capabilities into one relatively simple image transducing surface that would permit variable storage rate displays utilising television techniques.

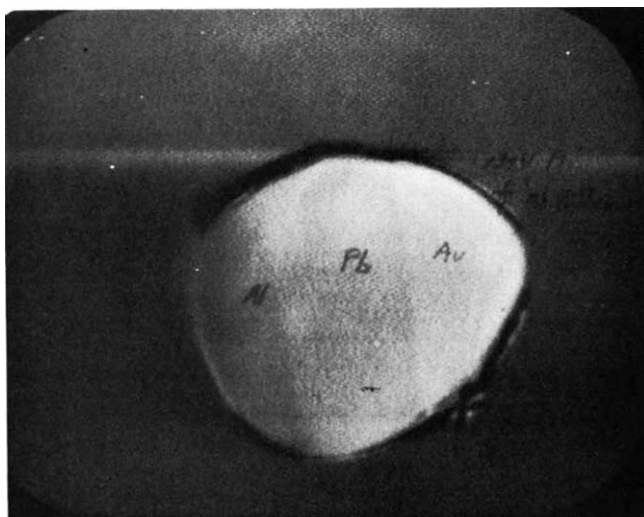


Fig. 1 Variation in sensitivity of metallonicon with differing metal converting layers. The Pb and Au layers are both 6 μm thick. Al is the uncoated signal plate. Note the improved performance of the system obtained when using the Au layer in combination with the CsI storage and signal enhancing layer. The X-ray generator used produces 75 kV potential self-rectified with 130 V applied to the primary. X-ray tube current was 5×10^{-4} A. Tube to target distance was 300 mm. Filter in beam 8 mm aluminium. Target diameter 100 mm. Image display rate 30 frames per second.

At penetrating-radiation energy levels exceeding 150 kV potential, heavy metal intensifying screens are used in preference to, or in combination with, fluorescent screens to provide increased speed and detail resolution for the radiographic process^{2,3}. Attempts have been made in the past to utilise this image forming technique in the construction of penetrating-radiation image converters⁴. These have, in general, not been of great practical consequence because of the extremely

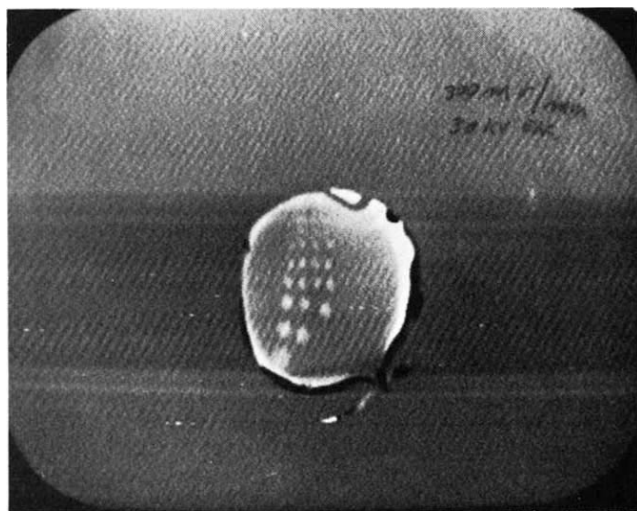


Fig. 2 Resolution obtainable at measure dose rate of 5 mr. s^{-1} at face plate of tube. Object is Pb sheet with holes ranging in size from 5 mm at bottom of image to 1 mm at top. Magnification of object detail due to X-ray generator-object-image target geometry. Diameter of image target 100 mm. X-ray generator 60 kV potential self-rectified. X-ray tube current 2×10^{-3} A. 25.4 mm aluminium placed between object and image tube target to achieve level and quality of radiation noted. X-ray tube to image target distance 300 mm. Image display rate 30 frames per second.

low yield of the absorbed quanta to photoelectron process and the relatively short image integration time.

Secondary electron enhancing materials such as KCl and CsI are routinely used as the image storage layer in television camera tubes capable of operation at the low light levels encountered in astronomical studies⁵. The tube reported here combines on one scanned surface the high energy conversion method of radiography with the storage and image enhancing features of the SEC target. Even at its present stage of development it exhibits characteristics that indicate it has potential value in many applications concerned with the imaging of high energy penetrating radiation fields.

This new image tube combines a heavy metal conversion layer (Au, Pb, or Pt) with a high resistivity secondary electron enhancing layer (CsI) to form the target of a television camera storage tube. The target of the tubes constructed so far is made using an aluminium substrate covered with an evaporated layer of gold about 6 μm thick upon which a layer of low density CsI is deposited. The low density CsI layer, prepared by evaporating CsI in an argon atmosphere of approximately one torr, has a mass per unit area of roughly 4.5 g cm^{-2} . When a field is established between the aluminium signal plate and the back surface of this layer by the scanning process, secondary emission is enhanced due to pores in the CsI layer. The electron gain obtained with these low density layers is higher by some 40 times than those obtained using bulk CsI.

Our work indicates that it is necessary to have a particular heavy metal emitting layer produce the photoelectrons. An experiment in which the heavy metal layer was both eliminated and varied in composition is illustrated by Fig. 1. Comparison studies between equal thickness Pb, Pt, and Au layers indicate that the Au layer is preferred in combination with the CsI film.

Tubes fabricated with 100 mm diameter Au-Cs targets have been tested utilising a half-wave rectified X-ray generator whose effective radiation energy was measured to be 35 kV potential. Response time of the tube when scanned at a rate of 30 frames per second was such that a moving object exhibited strobing due to the pulsating nature of the X-ray source. In other experiments, the scanning beam was used to charge the target to approximately 40 V potential and then turned off. The target was then exposed to 1.0 mr. of radiation during a 1 s exposure. Following a wait of 5 min, the scanning beam was turned on, at which time the image resulting from this 1 s exposure was seen as the charge pattern was equalised by the scanning beam. Figure 2 illustrates the images obtainable at incident dose rates of 5.0 mr. s^{-1} of radiation of 30 kV potential effective under continuous scanning at 30 frames per second.

The effective X-ray energy of 35 kV potential implies that the photoelectrons were ejected from the L-shell in Au. Continuing work indicates that by utilising the high energy photoelectrons and secondary radiation quanta from the K-shell in the energy range of 150 keV together with a micro-pyramidal substrate, a tenfold increase in sensitivity is obtainable.

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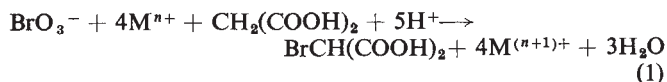
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Monomolecular treatment of chemical oscillation

THE mechanism of the Belousov oscillating reaction—the catalytic oxidation of malonic acid with bromate—is highly sophisticated. Although considerable progress has been made in revealing the kinetics and mechanism of both the complete oscillating system^{1,2}, and some of the composite reactions (especially those occurring between bromate and catalyst^{3,4}) many points need further investigation.

Looking at the temperature dependence of chemical oscillation and the role of different catalysts we thought to propose a simple treatment for the Belousov oscillating reaction. The basis for this lies in the fact—supported by early observations, and by our calorimetric and polarographic results—that the most important reaction in a Belousov oscillating system is the autocatalytic oxidation of the catalyst (M^{n+}) by bromate in the presence of malonic acid by the formation bromomalonic acid (BrMA);



In this phase of the oscillating reaction, the rate of heat evolution is very high, and this is synchronised with a sudden potential jump and decrease in bromide concentration⁵. This phase is followed by one where ordinary redox reactions (such as the reduction of $\text{M}^{(n+1)+}$ by malonic acid, bromate–bromide reaction) proceed and the rate of heat evolution is low. The latter phase can be regarded as a comparatively 'restful' one. When the bromide concentration in the system falls below a critical value ($\sim 3.10 \cdot 10^{-6}\text{M}$) (ref. 1), the autocatalytic reaction is switched on again. These processes follow each other periodically. Thus, the Belousov oscillating reaction can be regarded as a series of autocatalytic reaction 'bursts' occurring with a certain frequency.

This consideration led us to treat chemical oscillation as a 'monomolecular' reaction. As a first order rate constant (k_ω) we shall use the reciprocal of the period time at initial concentrations. From k_ω values measured at different temperatures using the Arrhenius equation the 'activation energy' of chemical oscillation (E_ω) can be calculated (ω refers to chemical oscillation expressing its periodic character).

Reacting oscillating systems with three different catalysts (Ce^{3+} , Mn^{2+} and $\text{Ru}(\text{dipy})_3^{+2}$) were investigated at 15, 25 and 35° C. The oscillating systems contained malonic acid (0.4 M), potassium bromate (0.1 M), sulphuric acid (1 M) and a catalyst (0.005 M) (system 1). From the frequency of the second (or third) period of chemical oscillation the activation energy of the oscillating reactions was calculated. The frequency changes with time because of concentration changes in the reactants, so it is necessary to choose an early period. Figure 1 shows the $\log k_\omega$ against $1/T$ plots. From Fig. 1 it is obvious that the Arrhenius equation is valid in the temperature range investigated and that E_ω is practically identical with all the three systems. $E_\omega = 16.1 \pm 0.2 \text{ kcalorie mol}^{-1}$ ($67.5 \pm 0.8 \text{ kJ mol}^{-1}$).

Using the absolute rate equation, the 'entropy of activation' of the oscillating reactions (ΔS_ω^*) was also calculated. Actually, ΔS_ω^* is the probability of 'autocatalytic reaction bursts'. Its value is $-12.5 \text{ calorie mol}^{-1} \text{ deg}^{-1}$ ($-52.5 \text{ J mol}^{-1} \text{ deg}^{-1}$) with all three catalysts.

We conclude that neither E_ω nor ΔS_ω^* depends on the nature (for example, the redox potential) of the catalyst. This identifies the chemistry of the 'burst' reaction (1).

Oscillating systems with different initial concentrations were also investigated. In a chemical system containing malonic acid (0.4 M), potassium bromate (0.1 M), sulphuric acid (0.5 M) and cerium (III) or manganese (II) (0.005 M) system 2 ($16.5 \pm 0.2 \text{ kcalorie mol}^{-1}$) $69.1 \pm 0.8 \text{ kJ mol}^{-1}$ was obtained for E_ω which is in a rather good agreement with the value obtained for system 1. k_ω , and thus the ΔS_ω^* , values, however, differ

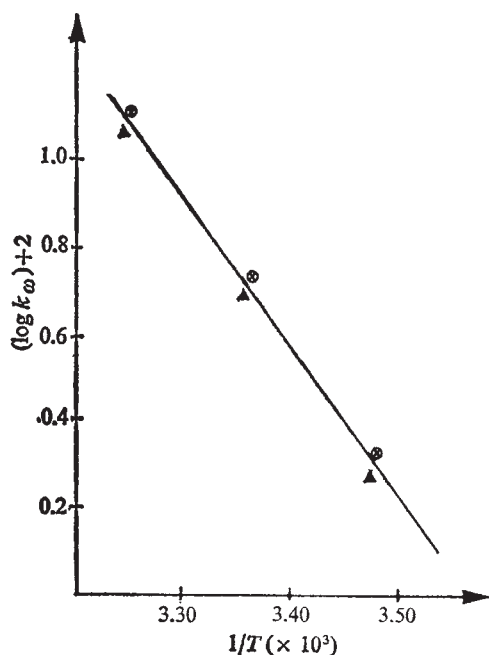


Fig. 1 Plot of the logarithm of the reciprocal of the period time ($\log k_\omega$) against $1/T$ ($T = 288,298$ and 308 K). The chemical systems containing malonic acid (0.4 M), potassium bromate (0.1 M), sulphuric acid (1 M) and catalyst (0.0005 M). $\circ$, Ce^{3+} ; $\times$ Mn^{2+} ; $\blacktriangle$, Ru(dipy)_3^{+2} .

with the two systems. (In system 1 $k_\omega = 0.053$ and in system 2 $k_\omega = 0.015$ at 25°C .) E_ω —at least within certain concentration ranges—is independent of the composition of the oscillating system the probability of ‘bursts’, however, is a function of the composition.

Are there any characteristics of the oscillating reactions which manifest themselves when one catalyst is replaced by another? An exhaustive answer cannot be given yet.

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Memory for words

LISTENERS do not ordinarily retain the syntax of a sentence for longer than is necessary to grasp its meaning; they rapidly forget both superficial ‘surface structure’ and underlying ‘deep structure’¹⁻³. What they retain is the sense of the sentence, evidently divorced from its syntax. It has been argued that this sense might consist of an associative structure linking representations of the words in the sentence⁴. Yet, if meaning is divorced from syntax, it might also be divorced from words. Indeed, we shall argue that a semantic representation need not incorporate any direct information about the lexical categories of words; it merely appears to do so because they can often be reconstructed from the meaning of a sentence. It follows, of course, that the specific wording of a sentence should ordinarily be

rapidly forgotten⁵. One simple test of this hypothesis, avoiding the predictability of words from meaning, is to examine a person’s ability to recollect whether a certain element of meaning was conveyed by a noun or a verb. (Does the reader recollect with any confidence whether, for example, we wrote earlier, ‘the divorce of meaning from syntax’, or ‘meaning is divorced from syntax’?) We predicted that information about lexical categories would tend to be forgotten if subjects were unaware that their memory for a sentence was to be tested, but that it would tend to be remembered if subjects expected their memory to be tested.

Two independent groups of subjects were told that their task was to make up two sentences continuing the theme of a short spoken passage. The members of the ‘memory’ group (M) were also told that they would be given a memory test for the passage; the members of the ‘incidental’ group (I) were given no such instruction. After listening to the tape recorded passage, which described a brief incident in a science-fiction story, both groups were given recognition tests for two of the sentences in the passage.

To test the hypothesis, we obviously had to use sentences whose meanings do not allow the lexical categories of their constituent words to be easily reconstructed. The third sentence of the spoken passage was therefore one of the following items:

- (1) The owner of the magic staff dispatched the ship.
- (2) The dispatcher of the ship owned the magic staff.
- (3) The owner of the ship dispatched the magic staff.
- (4) The dispatcher of the magic staff owned the ship.

The first pair of sentences are very similar in meaning—what one presupposes the other asserts—yet they convey their meanings by way of totally dissimilar lexical categories, for example, the information conveyed by the verb ‘dispatched’ in sentence (1) is conveyed by the noun ‘dispatcher’ in sentence (2). The second pair of sentences stand in a similar relation to one another, but their meanings are quite different to the meanings of the first pair of sentences: in the first pair it is the ship that is dispatched, in the second pair it is the magic staff that is dispatched. The fifth and final sentence of the spoken passage was selected from the following set of sentences constructed according to the same principles:

- (5) On earth, the interrogator of the Martians advised the President.
- (6) On earth, the adviser of the President interrogated the Martians.
- (7) On earth, the interrogator of the President advised the Martians.
- (8) On earth, the adviser of the Martians interrogated the President.

The sense of the remaining sentences in the passage was sufficiently flexible to accommodate any combination of the different versions of the third and fifth sentences. Thus, four different variations of the passage were recorded involving the following combinations of sentences: (1) and (6), (2) and (8), (3) and (7), and (4) and (5).

After listening to the passage, each subject was given a recognition test for the third sentence followed by a recognition test for the fifth sentence. These tests involved presenting the four versions of each sentence, together with their equivalents in the passive voice; both of the two sets of eight sentences were presented in different randomised orders. The subjects, 16 students at Sussex University, were assigned in rotation to a group and to one of the four passages. They were tested individually; and they were allowed, if necessary, to make three attempts in each test to select the actual sentence that had occurred in the passage.

There was no reliable difference between the groups in their ability to retain the correct meanings of the sentences: two subjects out of eight made semantic errors in group M, and four subjects out of eight made semantic

errors in group I. The crucial distinction between the groups concerned those syntactic errors that reveal an inability to remember the lexical categories of the original sentence even though its correct meaning has been retained, for example, the selection of sentence (2) where the original was, in fact, sentence (1). Only one such error was made by a subject in group M, whereas six subjects produced a total of eleven such errors in group I; the difference in the number of subjects making errors in the two groups was reliable ($P=0.02$, Fisher Yates exact test). When a subject makes two semantic errors his other attempt is likely to be a guess even if it is correct in meaning, so we excluded the data of two such trials from the totals although their inclusion does not alter the basic pattern of the results.

A second experiment with a similar design but different materials examined the ability of a further twenty-four subjects to remember such sentences as:

- (9) The borrower of the money purchased the car and the seller of the fridge bought the lawn-mower.
- (10) The purchaser of the car borrowed the money and the buyer of the lawn-mower sold the fridge.

This task proved to be more difficult than remembering the sentences of the first experiment. The subjects in group I made a total of twenty-two semantic errors and the subjects in group M made a total of eight semantic errors; and the difference between the groups was reliable ($S_n=73.25$, $P<0.015$, Mann-Whitney test). Yet every subject made at least one error symptomatic of a failure to retain lexical categories, for example, confusing sentence (9) with sentence (10).

The results of the experiment suggest that even when listeners successfully recall the meaning of a sentence they can only remember whether an element of this meaning was conveyed by a noun or a verb if they know that they are to receive a memory test. They are then liable to implement a method for retaining verbatim information. Otherwise, lexical categories appear to be rapidly forgotten along with the superficial and underlying syntax of the sentence. One could defend the thesis that sentences are remembered by forming an associative structure linking representations of their nouns and verbs by arguing that the associative machinery deals with nominals derived from verbs in a very similar way to verbs themselves. But this argument runs the risk of rendering the thesis untestable. It is precisely such items that prevent the automatic reconstruction of lexical categories from the meaning of a sentence. One could also defend the thesis that at least word stems are remembered, since it was only the suffixes of words which were manipulated (together with the syntax of the sentence). But this view is incompatible with the results of previous experiments^{2,3}. We prefer to think that meaning is something that can be put into words with a suitable syntactic structure rather than a direct representation of the structured set of words itself. The words and their structure can be thrown away as soon as their meaning has been grasped. If this thesis is correct, it is misleading to talk of a memory for the nouns and verbs of a sentence unless one is concerned with a deliberate attempt to memorise them. It is similarly misleading to talk of a memory for the underlying subject and object of a sentence, or for its underlying cases. Such terminology is tied to the words that occur in a sentence and, as we have shown, it can be misleading to talk of a memory for words.

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Genotype interaction with undernutrition and external environment in early life

IN spite of the well documented effect of early undernutrition on body and brain size in adult animals¹⁻³, its reported influence on behaviour has been less consistent. Changes in performance in learning tasks and activity tests have been found following undernutrition in early life⁴⁻⁷. Several studies, however, have shown little or no effect of such a manipulation⁸⁻¹⁰. Here we consider two factors which may contribute to variability in later behavioural response to early life undernutrition.

Recent work indicates that the type of environment in which animals are reared may modify the behavioural consequences of undernutrition¹¹⁻¹³. Subtle changes in rearing conditions in different laboratories might account for the apparent lack of effect of undernutrition in some cases and its significant influence in others. Another important variable which could account for variability in results is the genetic background upon which nutritional treatments are imposed. Thus, the effects of undernutrition during the period of rapid development may differ according to the particular genotype selected for study.

We examined the interaction of genetic background with both early life undernutrition and differential environmental conditions. The C57BL/6J and DBA/2J mouse strains were selected for our experiments because they differ genetically in the control of a large number of behavioural, biochemical and physiological characteristics¹⁴.

Mice were raised in three different environments and two levels of dietary protein. Five males and five females from each mouse strain were exposed to each of the above conditions.

Litters from dams of the C57BL/6J and DBA/2J mouse strains which had been previously mated in our laboratory were reared after parturition in *ad libitum* or undernourished nutritional conditions in either enriched, normal or impoverished environments. The enriched condition consisted of a large, clear, lucite box (75×40×20 cm high) divided diagonally in two, containing various manipulanda; the normal condition was represented by opaque, plastic home-cages (30×13×12 cm); the impoverished group was reared in sound-deadened, ventilated chambers in normal home-cages but with sensory input restricted by covering the top of the cages with a white painted, fitted metal sheet. Control or undernourished animals were fed a 27% or 8% casein diet respectively, containing 10% fat with minerals and vitamins added. The diets were made isocaloric by the addition of dextrose and contained 0.2% saccharin to improve palatability. The litters were culled to four animals on day 4 of life. After removal of the mother at 21 d, the weanlings remained in the appropriate environmental and nutritional treatment groups until 42 d after birth. At this age, they were separated by sex and maintained in normal laboratory conditions with food and water available *ad libitum* until behavioural tests were carried out 90-124 d of age.

At this time, animals were exposed to an illuminated (30 foot candles) Lehigh Valley photactometer, 45 cm in diameter, with a solid wood floor painted white, for 2 min on three consecutive days. The frequency with which animals intersected the light sources activating the photocells was recorded automatically every 30 s. Rearing and defaecation frequency were scored by an observer for each daily trial. Two days after the final open-field test, animals were exposed to a 'novel-object' test in the same apparatus. The number of visits to,

Table 1 The results of open-field and 'novel object' tests administered to mice of the C57BL/6J and DBA/2J strains 90–124 d of age fed either an 8 or 27% casein diet for the first 42 d of life

Test	C57BL/6J		DBA/2J	
	Normal (<i>n</i> = 25)	Undernourished (<i>n</i> = 28)	Normal (<i>n</i> = 30)	Undernourished (<i>n</i> = 28)
Mean daily open-field activity (counts per 30 s period)	19.86	16.19	23.26	23.24
Mean daily 'novel object' defaecation frequency	1.93	2.70	4.03	3.53
Mean daily open-field rearing frequency	2.94	2.01	6.87	6.24

Means for replacing missing data were utilised in seven cases in the C57BL/6J plus two cases in the DBA/2J strain. The effect of these data substitutions were taken into account in the calculating degrees of freedom, error terms and *F* ratios. Separate analyses of variance of each measure were carried out within each mouse strain. In the C57BL/6J strain, undernourishment reduced activity ($F = 3.90$; d.f. 1, 51; $P < 0.06$) and rearing frequency ($F = 4.05$; d.f. 1, 48; $P < 0.05$) in the open-field test. In the 'novel object' test, undernourishment significantly increased defaecation scores ($F = 4.19$; d.f. 1, 49; $P < 0.05$). Early life undernutrition had no overall effect in the DBA/2J strain.

and the time spent in the centre square containing a black spring mounted on a lucite base were recorded for a single 5 min test session. Total activity and number of defaecations were also recorded.

Analysis of variance was used to evaluate the main effect and interaction of early life undernutrition and environment and sex within each mouse strain. Where measures were repeated either within daily sessions or on consecutive days on the same animals, appropriate partitions of variance were also made¹⁵. This statistical treatment was applied to the three open-field test measures and the four 'novel-object' test scores¹⁵. Rearing and defaecation scores were transformed by a $\sqrt{x+0.5}$ transformation to normalise their distribution.

Females defaecated significantly more than males in the open-field test in both strains ($F = 11.17$; d.f. 1, 48; $P < 0.001$ in C57BL/6J strain; $F = 5.18$; d.f. 1, 46; $P < 0.05$ in the DBA/2J strain), but on other behavioural measures, no sex differences were found. Higher-order interactions involving sex were not consistent, easy to understand or significant at a very high level and, for this reason, data from both sexes are considered together.

In the C57BL/6J strain, early life undernutrition significantly reduced rearing frequency and tended to reduce activity, although the probability level associated with this effect was not very satisfactory ($P < 0.06$). It also increased defaecation scores in the 'novel-object' test. In contrast, the statistical analysis revealed no overall effect of nutritional restriction on any of the seven measures derived from the two tests in the DBA/2J strain. Table 1 shows the effects of early life undernutrition in the two strains on those measures which discriminated undernourished from control groups in the C57BL/6J strain.

In contrast to the effects of dietary restriction, the external environment for the first 42 d of life substantially influenced mice of the DBA/2J strain. On five out of seven behavioural measures, a significant effect of environmental manipulation was observed in the DBA/2J strain. Environmental impoverishment reduced open-field activity and rearing frequency. It also reduced activity levels and visits to the centre square and increased defaecation scores in the 'novel object' test.

By comparison, the only statistically significant effect of early

life environment in the C57BL/6J strain was in the 'novel object' test where impoverishment reduced the frequency of visits to the centre square. Table 2 depicts the effects of the manipulation of environmental conditions in early life in the two strains. Although the behavioural responses of the 2 strains to early nutritional restriction differed, effects on body weight at the time of testing were almost identical. Undernourished animals were 77% and 78% ($P < 0.001$) of control animal weights in the two strains.

Although no effect of undernutrition was detected in the DBA/2J strain when all experimental conditions were combined, undernutrition did consistently affect behaviour of DBA/2J mice raised in the normal environment. The interaction of environment $\times$ diet $\times$ time for open-field activity was statistically significant ($F = 2.938$; d.f. 6, 136; $P < 0.025$) in the DBA/2J strain. Undernutrition was correlated with reduced activity in DBA/2J mice raised in the normal environment, the effect being restricted to the first minute of the 2 min test. In contrast, there was no consistent effect of undernutrition in DBA/2J mice raised in the enriched or impoverished environments on this measure. A second statistically significant interaction of environment with diet ($F = 3.8$; d.f. 2, 46; $P < 0.05$) indicated that, if DBA/2J mice were raised in the normal environment, undernourished animals defaecated more than controls in the 'novel object' test. By comparison, nutritionally deprived animals raised in the enriched or impoverished environments defaecated less than controls. Therefore, although undernutrition had a greater effect in the C57BL/6J strain, the occurrence of these interactions in the DBA/2J strain emphasises the relative nature of the dependence of nutritional and environmental influences on genotype.

Our results which demonstrate genetic influences on behavioural response to both early life undernutrition and environmental manipulations should be considered in the light of previous discussions of gene-environment interactions in these mouse strains^{16–18}. Furthermore, the fact that genetic factors can influence an organism's response to undernutrition suggests a possible explanation of why undernutrition has been shown to influence some human groups but appears to leave others unaffected¹⁹.

Table 2 The results of open-field and 'novel object' tests administered to mice of the C57BL/6J and DBA/2J strains 90–124 d of age raised in environmental enrichment, normal home-cage conditions or environmental impoverishment.

Test	C57BL/6J			DBA/2J		
	Enriched (<i>n</i> = 14)	Normal (<i>n</i> = 20)	Impoverished (<i>n</i> = 19)	Enriched (<i>n</i> = 19)	Normal (<i>n</i> = 20)	Impoverished (<i>n</i> = 19)
Mean daily open-field activity per 30 s	18.40	17.72	17.95	25.05	24.76	19.93
Mean daily open-field rearing frequency	2.47	2.38	2.58	8.97	6.82	3.88
Mean novel-object activity scores	148.6	131.96	121.85	273.85	202.05	143.40
Mean novel-object defaecation frequency	2.05	2.40	2.50	3.10	3.45	4.80
Mean daily novel-object visits	0.88	0.61	0.39	1.28	0.75	0.35

Impoverishment reduced activity ($F = 3.53$; d.f. 2, 46; $P < 0.05$) and rearing frequency ($F = 6.73$; d.f. 2, 46; $P < 0.01$) in the open-field test in the DBA/2J strain. In the 'novel object' test, impoverishment decreased activity ($F = 7.25$; d.f. 2, 46; $P < 0.005$) and visits to the centre square ($F = 3.52$; d.f. 2, 46; $P < 0.05$) while increasing defaecation scores in the same situation ($F = 4.6$; d.f. 1, 46; $P < 0.025$) in this strain. In the C57BL/6J strain the only statistically significant effect of environmental manipulation was visits to the centre square in the 'novel object' test, where impoverishment reduced the frequency of visits ($F = 3.58$; d.f. 2, 50; $P < 0.05$).

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The earliest conifer

SMALL leaves and a leafy shoot from the Low Westphalian B (Upper Carboniferous) near Wakefield, Yorkshire, represent the oldest direct evidence for the existence of the order Coniferales (conifers) yet discovered. This material is preserved both as compression fossils and as charcoal.

Unlike most other fossil plants from the Coal Measures these leaves show both epidermal characters and internal structure. Most of the leaves are small (1–5 mm long), narrowly triangular (Fig. 1a) and are occasionally forked (Fig. 1b), similar in general appearance to Florin's genus *Lebachia*¹. The leaves are hypostomatic and have a denticulate margin (Fig. 2). The lower epidermis has two wide stomatal bands with closely spaced stomata which often share subsidiary cells and are monocyclic. The leaves are uninerved and the non-stomatal areas of both the lower epidermis and all of the upper epidermis consist of elongate cells interspersed with smaller cells. The outer (periclinal) wall of the epidermal cells (presumably including both cuticle and underlying cellulosic wall) is very thick (Fig. 3); this seems to have been burnt off in some of the leaves. A layer of fibrous tissue immediately underlies the epidermis, while mesophyll tissue is generally less well preserved. The nature of the vascular trace is ambiguous but it is believed that the tracheids have annular or scalariform thickenings.

This earliest record of conifers has both evolutionary and environmental significance. It is suggested that the leaves have been charcoaled by forest fire and have been

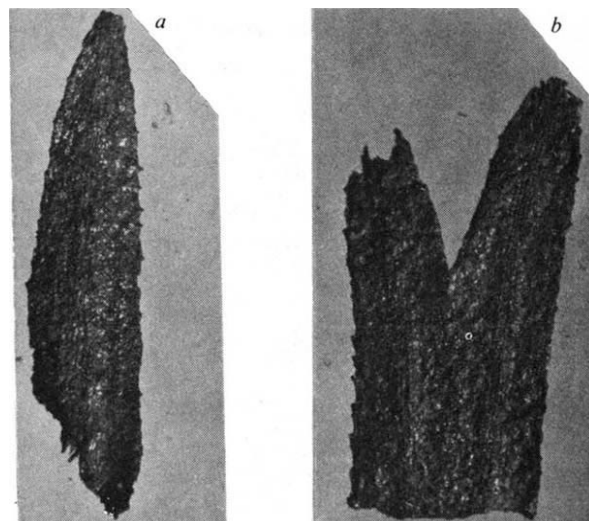


Fig. 1 Light micrographs of charcoaled conifer leaves. a, Small needle-like leaf ($\times 30$); b, forked leaf ($\times 30$).

fragmented during transport to their site of deposition. Many of the leaves show typical features of rapid burning such as collapse of epidermal cells (Figs 2 and 4), occasional absence of cuticle and the three-dimensional nature of the leaf—the cell lumina still remaining open (uncrushed; Fig. 3). The early conifers are rare, previous records coming from the Westphalian D (high Upper Carboniferous) and above. These all belong to the broadly based form genus *Walchia* Sternberg which Florin subdivided into two genera, *Lebachia* and *Ernestiodendron* on differences in their leaf arrangement, reproductive structures and cuticular characters. This new material has not been assigned to a genus as yet but is believed, from the information available, to belong to the family Lebachiaceae. No reproductive structures have yet been found but it is believed that the information obtained from the vegetative material is sufficient to justify inclusion in both the order Coniferales and the family Lebachiaceae. This will be discussed in detail elsewhere.

Such early conifers have not been found commonly in the fossil record presumably because their environment of growth differs from those plants normally found in the Coal Measures². The depositional environments in the Stephanian (high Upper Carboniferous) and Permian, especially in Europe, favoured the preservation of such plants.

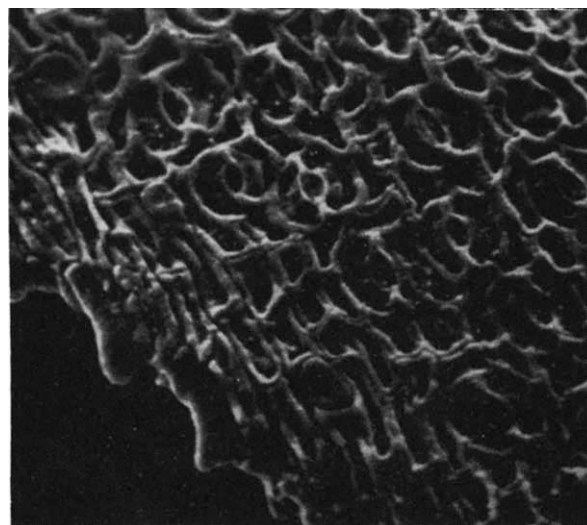


Fig. 2 Scanning electron micrograph of part of the lower epidermal surface of a leaf showing a stomatal band and a denticulate margin ($\times 465$).

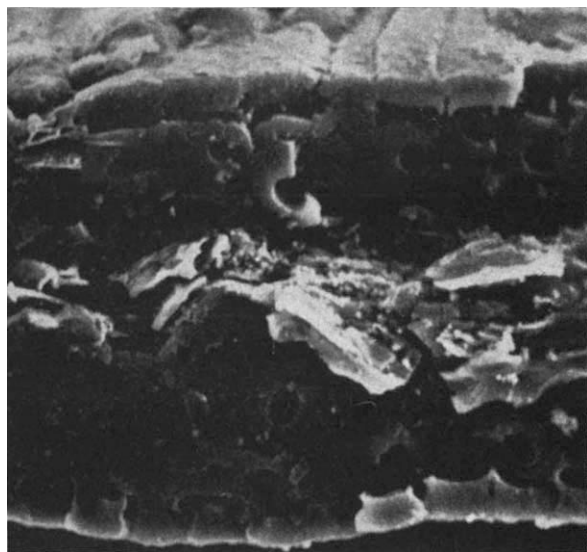


Fig. 3 Scanning electron micrograph showing part of the cross section of a typical leaf (thick cuticle, subepidermal fibres and crushed mesophyll tissue) ($\times 900$).

Pollen called *Potonieisporites* Bharadwaj has been shown to belong to the genus *Lebachia* Florin in the Permian⁷. Pollen of this type is also known from the Upper Carboniferous (Westphalian and Stephanian). It is now evident that such pollen may have been produced by conifers.

The discovery of conifer material, by bulk maceration of thin clay bands containing fusain within a poor coal, is important to the understanding of Upper Carboniferous floras as a whole. There is growing evidence that a fairly substantial flora is present in a charcoaled state, possibly of plants living associated with the conifers, and this may give insight into the palaeoecology of these (?hinterland) floras.

Harris⁴ has discussed forest fire in the Mesozoic and others (A.S., and de Klerk, unpublished) have considered it as a fossilising mechanism in the Tertiary. It is not surprising, therefore, that forest fire may have played an important role in the Upper Carboniferous and the nature of fusain bands within coals and shales must again be open for debate. Early records of conifers in the form of such

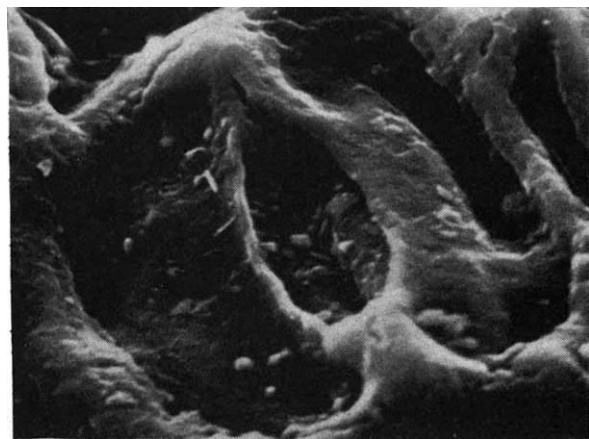


Fig. 4 Scanning electron micrograph of a single stoma showing upstanding anticlinal walls and collapsed guard cell walls. (An alternative explanation is that the guard cells are sunken below the surface and exposed only around the stomatal pit at the centre and that there are only two subsidiary cells which have themselves collapsed.) ($\times 4,500$).

charcoaled fragments may now be sought using the bulk maceration technique in the hope of learning more of their origins and ecology.

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A model for stably inherited environmentally induced changes in plants

THE phenotypes of some inbred lines of *Linum usitatissimum* (flax) and *Nicotiana rustica* can be altered environmentally by fertiliser treatments, and the fertiliser-induced phenotypes show stable inheritance independent of the inducing conditions¹⁻³. In flax, the stably inherited large (L) and small (S) phenotypes can be induced from the plastic (Pl) genotype *Stormont Cirrus* by one generation of growth with high nitrogen or phosphorus fertiliser treatments respectively. The induced phenotypes of L and S, differing for example in plant weight and degree of branching, behave in most respects as distinct genotypes and have been termed genotrophs.

Current genetic theory offers at least two explanations for such changes; biological examples of both types are examined in relation to the flax system.

(1) Informational nucleic acid may be introduced into flax under the inducing treatments, and be maintained in subsequent generations. Several groups⁴⁻⁷ have shown that exogenous DNA can be taken up, expressed and maintained by plants, though whether it is integrated into the host genome is uncertain. In flax, possible nucleic acid donors could be among the soil flora. Under the inducing treatment the soil flora may change, or conditions for uptake or incorporation of nucleic acid may be favoured. It could be established if flax DNA contains sequences hybridisable to DNA extracted from soil flora. This idea predicts that the induced changes should not occur with flax grown under sterile conditions.

Changes in the DNA content of the flax genotrophs do occur^{8,9}, but their relevance remains uncertain because the changes can be reversed without loss of either the induced phenotype or its stable inheritance¹⁰. The DNA changes are partly caused by variation in the redundancy of ribosomal DNA¹¹. It is unlikely that this change would be the primary event in genotroph induction, but could be a consequence of a regulatory switch such as described below.

(2) Some change in a regulatory circuit has been effected by the inducing conditions; this change persists after removal of these conditions, and involves no qualitative change in genome. Lysogens of bacteriophage λ show a temperature-induced loss of immunity in the host *Escherichia coli*, that is stably inherited independent of the inducing conditions with no change in the phage genome^{12,13}. λ prophage synthesises the *cI* gene product, the repressor protein, that renders the host *E. coli* immune to further homoimmune λ infection. *cI857* prophage has a temperature-

sensitive repressor protein active at 32°C but inactive at 42°C. *E. coli* containing prophage with genotype *cI857* N⁻ (O⁻ or P⁻) are immune at 32°C, but growth at 42°C inactivates the repressor and immunity is lost. At 42°C synthesis of the *cro* gene product (normally repressed by repressor) occurs and *cro* product prevents further repressor synthesis. On return to 32°C, *cro* product continues to prevent repressor synthesis, immunity is not regained and the immunity loss is stably inherited and dependent upon formation of the active *cro* product.

The λ system is analogous to genotroph induction and suggests a model for the induction and stable inheritance of the L and S genotrophs from the PI flax genotype. Figure 1a represents the regulatory system of the PI phenotype under non-inducing conditions; this system contains three regulatory proteins which act and remain in the flax cell nuclei and are coded on distinct structural

CP binds E^L to form a CPE^L complex inactive in controlling CP and AP synthesis, but still active in preventing IP synthesis. Consequently AP synthesis starts, CP synthesis stops and IP synthesis remains inhibited. AP triggers the L phenotype event sequence, inhibits CP and IP synthesis, and is a positive control element in its own synthesis. AP will now continue to be made in the absence of the inducing conditions (Fig. 1c), and the L phenotype will be stably inherited. A similar regulatory switch triggers the S phenotype from the PI one under inducing conditions. Here some effector E^S increases in concentration in the plant inactivating CP so that IP is synthesised which triggers the S phenotype event sequence. IP has analogous control properties to AP and the S phenotype is stably inherited.

The model explains other findings in the flax system. The F₁ progeny of an L × S genotroph cross are intermediate in plant weight, show high plant weight variance and are non-inducible by fertiliser treatments¹. In such a cross both zygotes contribute equal amounts of the nuclear regulatory proteins AP and IP; there are no maternal effects, and an unstable mix of both regulatory proteins each capable of inhibiting synthesis of the other occurs. In phage λ , the outcome of such a match between opposing gene functions can depend upon additional variables^{14,15}. In flax, individual plants should have a certain probability of changing from the mixed to the AP or IP regulatory mode. This could result in a wide population variance in plant weight, the extremes of which (sole AP or IP control) would be stably inherited. Similar explanations exist for the outcome of crosses that mix the CP and AP or IP regulatory proteins.

The primary sites of action of the AP and IP proteins could be modulation of plant hormone levels or regulation of synthesis of growth inhibitors. Determining the levels of plant hormones and growth inhibitors in PI, L and S plants seems a necessary step to biochemical analysis of induced changes.

The model predicts the properties of some mutations in the regulatory genes. A search for and genetic analysis of such mutants will both test the model and give more information on the nature of induced changes. Three pleiotropic loci, the structural genes for CP, AP and IP, should be identifiable and distinguishable from mutations in other genes altering components not directly related to the regulatory switch. Mutants in these loci should show stable L or S phenotypes independent of inducing conditions, or plastic phenotypes with altered inducibility.

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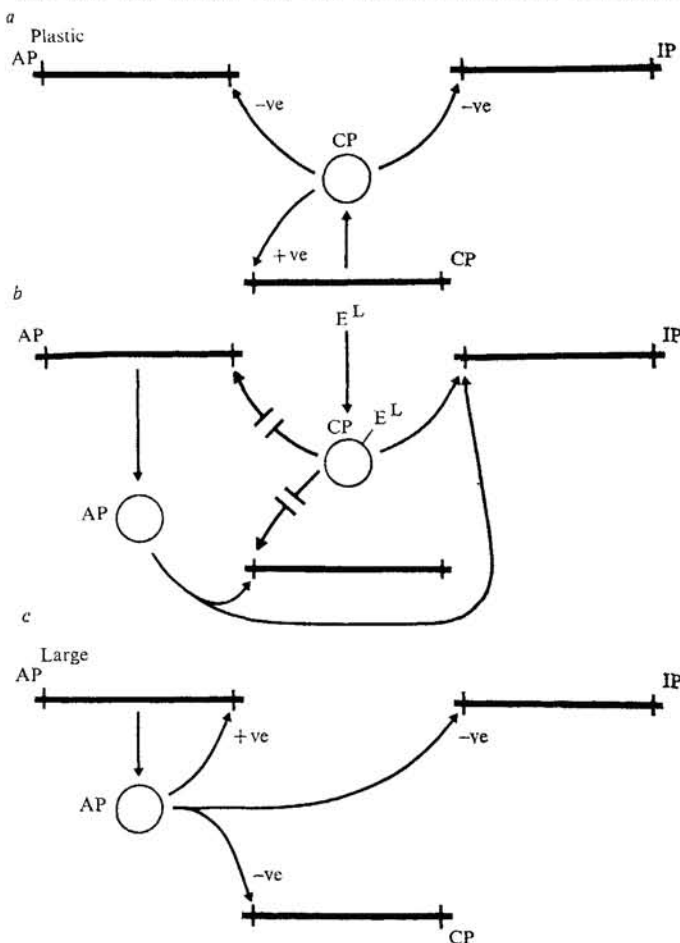


Fig. 1 Regulation of the PI phenotype and the induction and stable inheritance of the L phenotype in flax. The structural genes for the three regulating proteins are indicated by heavy lines; for simplicity the haploid situation is shown, the inbred lines being assumed isogenic. The regulating proteins are shown as circles and behave as transcriptional control elements.

genes. A control protein (CP) is made which controls its own synthesis in a positive manner. CP is also a negative control element preventing synthesis of two other regulatory proteins, an activator protein (AP) and an inhibitor protein (IP). AP and IP are involved in triggering sequences of events leading to the L and S phenotypes. The model is not dependent upon the precise mechanism of these events, though modulation of a plant hormone level may be involved. The site of CP regulation is shown as transcriptional, but could be translational. Figure 1b illustrates the switch from the PI to the L form under inducing conditions. High nitrogen fertiliser treatment increases the concentration in the plant of some effector molecule E^L.

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Control of food intake by energy supply

A WIDE variety of metabolic influences on appetite have been proposed, including glucose utilisation rate¹, influences from adipose tissue^{2,3} or plasma amino acid pattern^{4,5} and the heat increment during absorption of a meal⁶. It is difficult to relate short term effects of the metabolism of absorbed nutrients which indeed occur⁷, to the long term precision with which energy exchange is often regulated.

A direct signal from fat stores to the brain has been postulated³ but an alternative is the modulation of a short term 'glucostatic' signal by a 'lipostatic' signal⁸. A more general proposal along the latter lines is that the signal at the neural receptors controlling feeding in the omnivore reflects the current supply of energy for intermediary metabolism from whatever source, including sugars, amino acids, fatty acids, ketones, glycerol and lactate^{7,9}. There is some reason to believe that this metabolic control is primary in the control of food intake, with control by sensory and gastrointestinal mechanisms normally being adjusted according to metabolism¹¹⁻¹³.

The theory that current energy supply is the primary factor controlling appetite has now been incorporated for the rat in a computerised physiological control model illustrated in block diagram form in Fig. 1. The pattern of feeding is correctly predicted within the limits of accuracy of the physiological data which completely determine the computation (Table 1).

The rate at which the rat feeds is taken to be constant throughout a meal, as an initial simplification. A feeding rate of 1,000 calorie min⁻¹ (about 0.3 g min⁻¹) is an intermediate value from the literature^{14,15}. The rate at which the gut clears nutrient energy is taken to be proportional to the square root of the amount of energy contained in the gut, a function based on studies of gastric carbohydrate clearances^{9,16}. The constant of proportionality (K) is higher (0.9) by night than by day (0.6) in the rat, a nocturnal animal; this value is taken to change linearly over 2 h around dawn and dusk. These calculations predict the variations in rate of absorption of energy-yielding substances during and between meals.

Another source of variation in total energy flow is storage and mobilisation of fat. This has been based in the model on the results of Le Magnen and Devos¹⁷, which indicate mainly net subtraction of energy from fat store by day and net addition of fat by night.

Threshold values for initiation and termination of feeding purely on the basis of total energy flow were based on amounts found in the gut at the start and end of meals and on the effects of infusing glucose into the hepatic portal vein in the rat¹⁸. Feeding is initiated when energy supply rate falls to below 18 calorie min⁻¹. It is fully suppressed on a single diet when energy is supplied at or above 60 calorie min⁻¹. In this initial approximate model these estimated values are taken to be without statistical variation.

The complete system was programmed for a digital computer, using a method of successive addition: all variables were adjusted once every 10 s of real time. The simulation was run for 6 d of real time, to give the data of Table 1. All correlations, real and modelled, are associated with a probability of $P < 0.01$. Considering the simplified picture of the total energy flow and the considerable assumptions underlying the estimates of flow on which the calculation is based, the agreement with the food intake characteristics of real rats is reasonable. It may be seen that there is a significant correlation between the size of a meal and the time to the onset of the next meal. Correlations of meal size to premeal interval are smaller and less reliable statistically in both real and modelled rats.

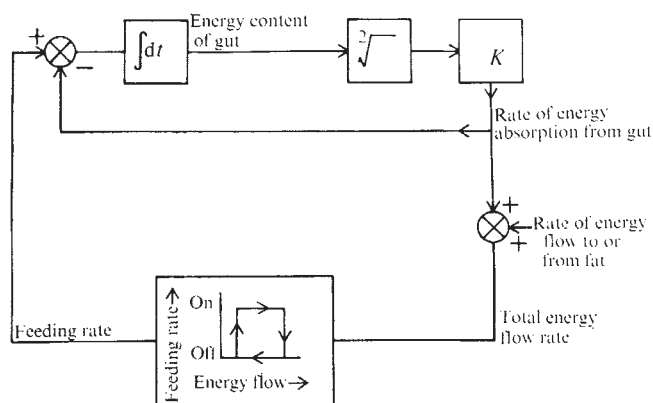


Fig. 1 A model of feeding.

The success of the simulation is not a confirmation of simple homeostatic concepts, for the model is based on rates of energy flow, not directly on amounts of energy eaten or stored. Further preliminary work suggests that the model can be extended to include estimates of increments in body energy which would link short term control to long term regulation.

Where the physiological data are available, conditions other than *ad libitum* maintenance of intact animals can be modelled. Indeed, an homologous model can be constructed for man. Hitherto, models of the control of food intake have not been based on physiological data¹⁹, have been unprogrammable and non-predicting block diagrams^{10,20-22} or have been computerised by postulating unmeasured variables and deciding parameters by fiat²³. The conditions simulated to date using the present model have generated realistic predictions. The dynamic and static phases of the hyperphagia which follows lesions of the ventromedial hypothalamus have been successfully simulated²⁴. This implies that the hyperphagia is secondary to hormonal abnormalities. The feeding pattern of the recovered lateral hypothalamic aphagic rat is mimicked by the model, if feeding rate is substantially slowed. This suggests that disruption of oral reactions is primary in the residual deficits of this condition.

The chronically diabetic rat has been modelled on the basis that in this condition there is no net storage or mobilisation of fat and only one-third of the ingested energy is able to act on receptors suppressing feeding^{25,26}. The model correctly predicts a food intake of about 60 g per 24 h. The real rat, however, takes the food in many meals distributed throughout the day and night²⁵ whereas the model of Fig. 1 predicts a single very prolonged meal in each lighting phase. A simple addition, that of an inhibitory pathway from stomach stretch receptors to the hunger system³ brings the model into agreement with reality.

Table 1 Feeding pattern parameters in real and modelled rats (means $\pm$ s.d.)

	A*	Real rat B†	C†	Modelled rat
Dark Phase				
Total intake (g in 12 h)	13.0	17.3 $\pm$ 3.4	14.2	13.3 $\pm$ 1.6
Size of meal (g)	2.6	3.1 $\pm$ 2.2	2.9	2.1 $\pm$ 0.4
Meal to meal interval (min)	137	160	—	100 $\pm$ 15
Meal size/post-meal interval: ratio	27.8	—	—	20 $\pm$ 3
correlation	—	0.84	—	0.76
Light Phase				
Total intake (g in 12 h)	8.5	5.1 $\pm$ 1.3	7.0	6.4 $\pm$ 0.9
Size of meal (g)	2.0	1.6 $\pm$ 1.2	2.3	2.2 $\pm$ 0.9
Meal-to-meal interval (min)	154	218	—	282 $\pm$ 73
Meal size/post-meal interval: ratio	14.3	—	—	9 $\pm$ 0.6
correlation	—	0.65	—	0.98

*Ref. 14

†Ref. 17

Even in the present single-factor theory of feeding control, many components interact. The verbal theories currently extant, and even intuitions concerning the predictions of quantitative theories such as the present one, can only be evaluated decisively by specification as a computer model.

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We devised a new, simple and reliable quantitative assay of the red cell ADA activity. With this method we found that the mean activity of the red cell ADA in the ADA 1 is higher than that of the ADA 2-1 phenotype. This shows that the activity associated with the *ADA*¹ allele is greater than that associated with the *ADA*² allele.

A random sample of 196 undergraduate students (first year) of the University of Rome was studied. The red cell ADA phenotypes were determined according to Spencer *et al.*⁴. The red cell ADA activities were all assayed within a few hours after bleeding. The present method is an adaptation to red cell lysates of Kalchar's method⁸. The lysates were incubated with adenosine in excess. The reaction was stopped with perchloric acid and the quantity of adenosine consumed was estimated from the decrease of $E_{2,680}$ (inosine and hypoxanthine have the same $E_{2,680}^{Mol}$ which is about half that of adenosine⁹. In our hands the $\Delta E_{2,680}^{Mol}$ of the transformation adenosine into inosine or hypoxanthine was 6.77×10^3). Linearities with time and haemolysate concentration were checked. Haemoglobin concentration was determined with the

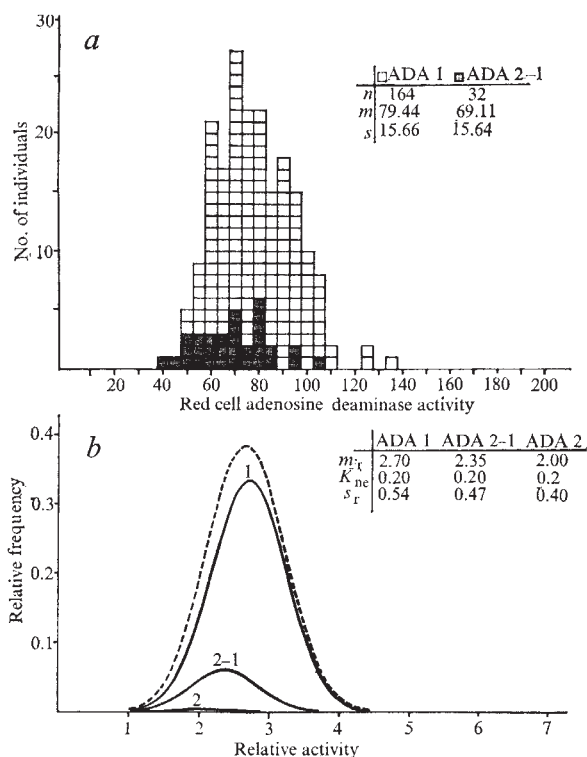


Fig. 1 Distribution of red cell adenosine deaminase activities. *a*, Observed distribution in a sample of 196 random individuals. *b*, Expected normal distributions in the ADA 2, ADA 2-1 and ADA 1 phenotypes with relative mean activities, m_r , 2.00, 2.35 and 2.70, respectively. The coefficient K_{ne} of intraphenotypic variation, that is of the non-electrophoretic variation, is assumed to be 0.20 for all the three phenotypes. The relative areas correspond to the expected relative genotype frequencies, $p^2=0.007$; $2pq=0.150$ and $q^2=0.843$.

Packed red cells washed three times with isotonic saline were lysed by a triplicate cycle of freezing (in acetone and dry ice) and thawing. The lysates were diluted 50 times with Sørensen phosphate buffer 1/15 M pH 7.5 preincubated at 37°C. The reaction was started within 15 min (the enzyme activity is stable in these conditions for at least 1 h) by mixing 1 vol of lysate with 5 vol of a preincubated solution of 0.337 mM adenosine in the same buffer. At 0 time and 75 min three aliquots were transferred into equal volumes of 8.7% PCA. After at least 15 min the precipitates were spun down and 5 ml of the clear supernatants were neutralised with 0.35 ml of 5 M K_2CO_3 , transferred into a freezer for about 10 min and filtered. The $E_{2,680}$ were then determined. Haemoglobin was determined in the assay mixture (adenosine+lysate).

The activity is expressed as μ mol of adenosine consumed per h at 37°C per g of haemoglobin.

Comparative activity of red cell adenosine deaminase allelic forms

CERTAIN enzymes have allelic forms which can be distinguished electrophoretically. The variability of the structural genes determining these enzymes¹ is one of the most challenging problems in modern biology, since no particular reason can be found for their selection during evolution and so they form the main subject of debate between the 'pan-neutralists' and the 'pan-selectionists'. To try and throw some light on this problem we have compared the enzymic activity of the different electrophoretic forms of human red cell adenosine deaminase.

Only a few enzymes have previously turned out to be suitable for this type of comparison^{2,3}—those with at least two common electrophoretic phenotypes and a simple quantitative assay.

Adenosine deaminase (ADA) presents an electrophoretically demonstrable polymorphism depending upon an autosomal gene with two common codominant alleles *ADA*¹ and *ADA*², as shown by Spencer *et al.*⁴. A number of cases of ADA deficiency have also been reported⁵. They were associated with a severe combined immunodeficiency disease⁵, and a causal relationship between the enzyme deficiency and the immunological disease mediated by the resulting pyrimidine starvation has been suggested⁶ (see also Giblett *et al.*⁷).

cyanmethaemoglobin method¹⁰. The precipitation of proteins makes this method more laborious but more sensitive (a more concentrated lysate may be used) and less expensive (no need of xanthine oxidase) than that of Hopkinson *et al.*⁹.

Figure 1 shows the distributions of the red cell ADA activities observed in the two ADA phenotypes found in the present sample.

The difference between $\overline{ADA\ 1}$ and $\overline{ADA\ 2-1}$ (the mean enzyme activities of the ADA 1 and ADA 2-1 phenotype, respectively) is statistically significant ($t=3.41$, $P<0.001$) in spite of the large intraphenotypic variation. The enzyme activity (expressed as μmol of adenosine consumed per h at 37°C per g of haemoglobin) associated with the ADA^1 allele, estimated as $(\overline{ADA\ 1})/2$, is 39.77, that is, 35% higher than that associated with the ADA^2 allele (29.34) estimated by subtracting the ADA^1 activity to $\overline{ADA\ 2-1}$, on the assumption that no interaction occurs between ADA^1 and ADA^2 alleles in the heterozygote. This seems to be a quite reasonable assumption, considering that no hybrid ADA molecules are present in the heterozygotes.

A portion of the variation of the red cell ADA activity can thus be accounted for in terms of the difference existing between the activities associated with the two common electrophoretically distinguishable alleles of this enzyme. This portion can be conveniently expressed as the corresponding partial variance, which is estimated to be, in our sample, 0.019 if one designates as 1 the activity associated with the ADA^2 allele (see Fig. 1). This variance is in fact equal to $2pq.d^2(1+K_{ne})^2$ (see Modiano³) where $p(ADA^2)=0.08$ and $q(ADA^1)=0.92$ are the gene frequencies found in the present sample (which are similar to those previously reported for the same population¹¹), d , the relative difference between the ADA^1 and ADA^2 activities, is 0.35 (see legend of Fig. 2); and K_{ne} , the coefficient of the non-electrophoretic variation (standard deviation divided by the respective mean within each electrophoretic phenotype), is rounded to 0.20 for all the three ADA phenotypes. Since the observed total variance was 0.282, it may be concluded that $0.019/0.282$, that is about 7% of the total variation of the red

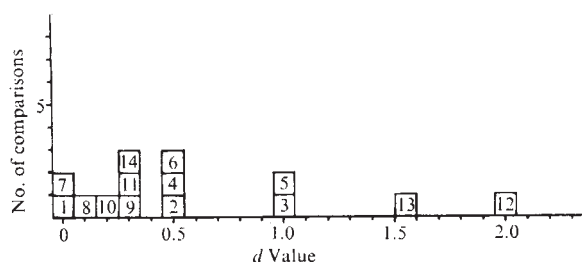


Fig. 2 The distribution of the 14 relative differences, d , known at present between the enzyme activities associated with common electrophoretic alleles of red cell enzymes*.

d is the relative difference between the enzyme activities associated with different alleles expressed in terms of the activity of the least active allele. For example, if the absolute allelic P^a , P^b and P^c activities are 60, 90 and 120 as in the red cell acid phosphatase, their relative activities are 1.0, 1.5 and 2.0, respectively, with three d values: $d_{P^b-P^a}=1.5-1.0=0.5$; $d_{P^c-P^a}=2.0-1.0=1.0$; and $d_{P^c-P^b}=2.0-1.5=0.5$. The comparisons refer only to those allelic enzymes whose associated activities have been compared because they were known to be electrophoretically different.

A list of the 14 comparisons. Each comparison is represented in the figure by the number in the corresponding box: acid phosphatase^{12,13}: (1) P^r-P^a , (2) P^b-P^a , (3) P^c-P^a , (4) P^b-P^r , (5) P^c-P^r , (6) P^c-P^b ; phosphoglucomutase^{14,15}: (7) $PGM_1^1-PGM_1^2$; glucose-6-phosphate dehydrogenase^{16,17}: (8) Gd^b-Gd^a ; Adenylate kinase^{18,19}: (9) AK^1-AK^2 ; 6-phosphogluconate dehydrogenase²⁰⁻²²: (10) Pgd^a-Pgd^b ; Glutathione reductase²³: (11) $GSSG-R^S-GSSG-R^F$; Glutamic pyruvic transaminase^{24,25}: (12) Gpt^1-Gpt^2 ; Uridine monophosphate kinase⁷: (13) UMP^1-UMP^2 ; Adenosine deaminase: (14) ADA^1-ADA^2 (this paper).

* See Modiano³ for detailed references.

† The difference in the figure is that reported by Chen *et al.*²⁴.

Table 1 Non-random assortment* of the relative activities and frequencies of the common alleles of red cell enzyme polymorphisms

	Polymorphisms in which the most active allele is	
	the most common allele	the least common allele
Observed	12†	1‡
Expected	6.5	6.5

* The likelihood of a discrepancy from randomness like the one observed or greater, in either direction, is $2(13+1) \times (1/2)^{13} \approx 0.003$.

† The following is the list of these twelve red cell enzyme polymorphisms: acid phosphatase^{12,13} (the findings in Caucasians where the most common allele is the one with intermediate activity have not been considered informative); glucose-6-phosphate dehydrogenase^{16,17}; adenylate kinase^{18,19}; 6-phosphogluconate dehydrogenase²⁰⁻²²; glutamic-pyruvic transaminase^{24,25}; galactose-1-phosphate uridyltransferase²⁶; nicotinamide adenine dinucleotide nucleosidase²⁷; phosphoglucomutase (PGM_2)²⁸; peptidase C²⁹; peptidase A^{30,31}; uridine monophosphate kinase⁷; adenosine deaminase (this paper).

‡ Glutathione reductase²³.

cell ADA activity, is associated with the electrophoretically demonstrable genetic variation of this enzyme. The nature of the remaining major portion of variation (the intraphenotypic or non-electrophoretic variation) remains unknown. It is also unknown why the ADA 1 and ADA 2 allelic enzymes present different activities in the red cell lysates and whether or not this difference applies to other tissues as well. These questions remain unanswered for most of the interallelic differences so far identified.

The difference in activity between the different allelic forms is a common finding. Figure 2 shows that, as a rule, common electrophoretic alleles are associated with considerably different ($d \approx 0.6$) enzyme activities in red cell lysates (12 out of 14 comparisons).

Further evidence suggesting that the common structural gene variations are not neutral is the non-random relationship between the relative frequency and activity found among the common alleles of the red cell enzyme polymorphisms studied so far (see Table 1).

In vitro V_{max} , however, is a poor measure of the enzyme's function *in vivo*. Significant progress along these lines may therefore be expected from interallelic comparisons involving more significant enzyme characteristics, such as K_m (in relation with the *in vivo* substrate concentrations) and the *in vivo* stability. But so far, only glucose-6-phosphate dehydrogenase has been studied at such a level³³⁻³⁶. The use of intact red cells under physiological or suitable stress (such as drug induced) conditions³⁷ or of other cells and tissues should also provide further rewarding information.

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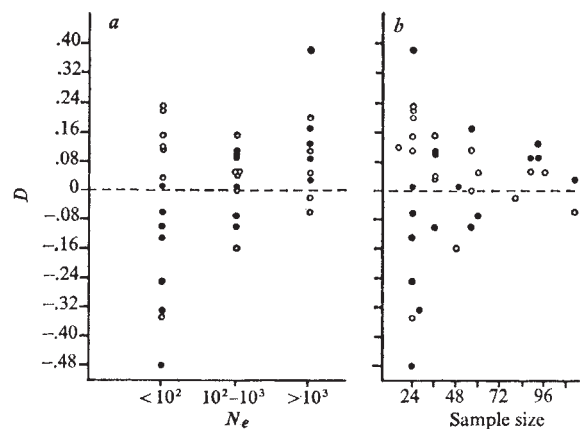


Fig. 1 *a*, Effect of adult population size (N_e) on heterozygote deviations (D) at the PGM-2 and LAP-2 loci in *C. nemoralis*. The zero line represents exact Hardy-Weinberg equilibrium where $D = H_o - H_e/H_e = 0$. Heterozygote deficiencies appear below the zero line, excesses above it. *b*, Effect of sample size on heterozygote deviations at the LAP-2 (○) and PGM-2 (●) loci. See text for further explanation.

sampling before completion of selection⁹ or inappropriate pooling of subpopulations. Although data on the size and spatial structure of the populations studied would often facilitate interpretations of genetic results, they are usually not available and with certain organisms are almost impossible to obtain⁷. I have now obtained such data for populations of the helicid land snail *Cepaea nemoralis* and found that heterosis maintains polymorphisms at two enzyme loci.

Because of their highly polymorphic shell patterns, populations of *C. nemoralis* have been subject to evolutionary study in Europe (see ref. 10 for a review) for decades. The species has been introduced into North America on several occasions, and colonies are now found in various localities in the eastern United States and Canada. They rarely occur outside towns or other habitats perpetuated by human disturbances. The colonies are either subdivided into small, local populations, or may occupy areas much larger than the estimated panmictic unit of the snails¹¹. For this reason, the snails provide excellent opportunities for studying the effects of spatial patterning and population size on genetic variation.

In addition to their shell colour and banding polymorphisms, *C. nemoralis* populations found in the Piedmont and Great Valley of Virginia are polymorphic at one phosphoglucomutase locus (PGM-2), and at one leucineamino-peptidase locus (LAP-2): PGM-2 has two alleles and LAP-2 usually has three. The enzymes provide clear electrophoretic patterns with good separation of all allelic variants. Thus, the possibility of scoring errors is remote. The genetic bases for the observed variation have been verified experimentally¹².

To study the effects of spatial patterns and effective population size (N_e) on genetic variability in *C. nemoralis*, 303 immature individuals 9 mm or more wide and 586 mature specimens were collected from 18 Virginia localities between June 1972 and October 1973. The area occupied by the snails was measured or estimated at each site.

Although *C. nemoralis* is monoecious, it is an obligate outcrosser. Therefore, the maximum N_e of a population approximates the numbers of mature individuals in that population¹¹. It is difficult to estimate the number of snails in natural populations since they move around too slowly for ordinary capture-recapture methods, and their patchy distribution makes quadrat or transect counts unreliable¹³. Small areas, however, could be searched completely, and estimates of N_e in these populations were obtained by direct count. Few mature specimens could have been

Population size and natural selection in the land snail *Cepaea nemoralis*

ALTHOUGH most investigators have concluded that protein polymorphisms are maintained by balancing selection^{1,2}, some contend that they are the consequences of random drift of neutral or nearly neutral alleles^{3,4}. Many empirical data seem to support the selectionist view, although the mechanism that might be involved is not well understood. In any case, critical data on changes in allelic or genotypic frequencies through time are not available for most natural populations. Therefore, the major evidence for selective responses is provided by deviations from expected zygotic proportions at some given time.

Studies of laboratory populations have indicated that some enzyme polymorphisms are examples of heterosis (overdominance), since experimental results consistently show heterozygote excesses over expected values^{5,6}. Data from natural populations are, however, harder to interpret^{7,8}. For example, heterozygote deficiencies can result from inbreeding or disruptive selection, or they can be an artefact caused by small sample sizes, scoring errors,

Table 1 Allele frequencies, heterozygote deviation, and sample size at the *LAP-2* and *PGM-2* loci in *C. nemoralis* from 18 local populations in the Piedmont and Great Valley of Virginia

		LAP-2					PGM-2					
		$f(a)$	$f(b)$	$f(c)$	D	Sample N	$f(a)$	$f(b)$	D	Sample N	N_e	
Lynchburg	1	0.47	0.07	0.46	-0.06	113	0.65	0.35	0.03	112	> 1,000	
	2	0.10	0.07	0.83	0.03	36	0.49	0.51	-0.10	35	< 100	
	3	0.24	0.01	0.75	0.15	36	0.44	0.56	0.11	36	100-1,000	
Staunton	1a	0.01		0.99	0	57	0.90	0.10	-0.10	57	100-1,000	
	2	0.31	0.06	0.63	0.20	24	0.71	0.29	0.38	24	> 1,000	
	3	0.21	0.04	0.75	0.05	60	0.79	0.20	-0.07	60	100-1,000	
	4	0.53	0.21	0.26	0.04	36	0.47	0.53	0.10	36	100-1,000	
Harrisonburg	2	0.11		0.89	0.11	56	0.49	0.51	0.17	56	> 1,000	
Lexington	1	0.27	0.01	0.72	-0.02	79	0.48	0.52	0.13	91	> 1,000	
	2	0.25	0.03	0.72	0.05	88	0.41	0.59	0.09	89	100-1,000	
	3	0.23	0.01	0.76	-0.16	47	0.48	0.52	0.01	49	100-1,000	
	4	0.33	0.04	0.63	0.23	24	0.60	0.40	-0.06	24	< 100	
	5	0.29	0.04	0.67	0.22	24	0.43	0.57	-0.13	23	< 100	
	6	0.25		0.75	-0.35	24	0.27	0.73	-0.48	24	< 100	
	7	0.17	0.25	0.58	0.15	24	0.44	0.56	-0.25	24	< 100	
	8	0.25	0.15	0.60	0.11	24	0.58	0.42	0.01	24	< 100	
	9	0.20	0.10	0.70	0.12	15	0.43	0.57	-0.33	27	< 100	
	10	0.33		0.67	0.05	95	0.22	0.78	0.09	93	> 1,000	
Total					862				884			

N_e is given for each population.

overlooked, and this procedure for determining *N_e* in small populations is probably less biased than any other.

Numbers of adult snails in the larger populations were estimated by a time-unit removal method. The principle is that if a known number of snails is removed from a designated area in a fixed time, the number found subsequently is affected. The rate at which catches fall off (a geometric series) is directly related to the size of the total population and the number previously removed¹⁴. By taking samples from relatively large plots (9 m²), I minimised the problem of temporary patchiness about single plants or other objects, and average density estimates of mature individuals were reasonably consistent from plot to plot within a population. Average numbers of adult snails per unit area were then multiplied by the total area occupied by the population to estimate maximum *N_e*. The size of the panmictic unit in large populations of *C. nemoralis* has been estimated to be more than 1,000 individuals¹⁵. My own unpublished data for North American populations confirm this.

I used the horizontal starch-gel electrophoresis techniques of Selander *et al.*¹⁶, modified as described before¹². Allele frequencies, determined by direct count from the phenotypes observed on the gels, were used to calculate the expected genotypic frequencies by Levene's¹⁷ method for small samples. Deviations of observed from expected values were tested for significance using the G test for goodness of fit¹⁸. For statistical analysis *LAP-2*^b was pooled with the second least common allele in most samples. There were departures from Hardy-Weinberg expectation in all cases except one (*LAP-2*, Staunton 1a; see below), though statistical significance was not noted in any.

Heterozygote deviation (*D*) was calculated by the formula

$$D = (H_o - H_e) / H_e$$

where *H_o* and *H_e* are the observed and expected numbers of heterozygotes, respectively. Table 1 lists the sample sizes, allele frequencies and values of *D* for both loci, and the estimated size of *N_e*.

At the *LAP-2* locus, 13 of 17 populations showed a heterozygote excess, which was unlikely by chance alone (*P*=0.025, sign test). (The eighteenth population, Staunton 1a, was not considered since the only variant in it was one *LAP-2*^{a/c} heterozygote.) This result does not conform to the hypothesis of neutral alleles, which would predict a statistically equal number of heterozygote excesses and

deficiencies. The simplest explanation for the heterozygote excess is some sort of balancing selection which results in some degree of superior heterozygote survival.

On the other hand, at the *PGM-2* locus seven of the 18 populations showed a heterozygote deficiency, indicating no overall tendency toward heterozygote excess (*P*=0.24, sign test). However, if the populations are grouped according to size, six of seven populations with *N_e* estimated to be less than 100 show heterozygote deficiencies, while nine of 11 populations with *N_e* greater than 100 show heterozygote excess. These differences from expectation are significant (*P*<0.025, Fisher exact test), and indicate a strong interaction between heterozygosity and population size (Fig. 1a).

No interaction is evident between sample size and the direction of *D*; although, as would be expected, values of *D* tend to have higher variances at lower sample sizes (Fig. 1b).

The findings suggest that the *LAP-2* and *PGM-2* polymorphisms are maintained in these natural populations by heterosis. Selection for heterozygotes may be stronger at the *LAP-2* locus, since heterozygote excesses are present even in populations with small *N_e*s. A possible explanation is that *LAP* catalyses the degradation of a diversity of substrates, some of which presumably enter the snail from the environment¹⁹. Individuals heterozygous at this locus may be able to utilise a greater diversity of energy or nutrient sources by virtue of having two forms of the enzyme. An alternate explanation is that this locus is closely linked to another which is subject to heavy selection.

PGM, on the other hand, has only been shown to catalyse the conversion of α-D-glucose-1-phosphate to α-D-glucose-6-phosphate. The apparently milder heterotic effects may result from favourable interactions with other gene products rather than from a capability to handle a greater variety of substrates. The validity of such speculations can only be established when the functional properties of the electrophoretic variants are understood. In any case, these data indicate that the effects of stochastic processes in small populations are strong enough to override any heterozygote advantage, either at this locus or at any closely linked to it.

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Alternate host for mosquito parasite *Coelomomyces*

THE water mould *Coelomomyces* (Chytridiomycetes, Blastocladales) is an obligate parasite of mosquito larvae. The fungus develops in the haemocoel of the host and is first visible as small unwallled hyphal bodies, which later differentiate into masses of thick-walled resistant sporangia. In appropriate conditions, each sporangium releases a large number of posteriorly uniflagellate zoospores¹. Field studies of this pathogen indicate that it may be responsible for significant epizootics of mosquito larvae and considerable interest has been generated over its possible use in biological control of these troublesome insects²⁻⁶. This potential has not been confirmed in the laboratory. The production of infected larvae in controlled environmental conditions has proved to be a difficult and erratic task^{1,7}. Couch⁸ has devised a technique for the production of large numbers of infected mosquitoes but this method uses quantities of microalgae and water from the original infection site.

Our studies have focused on *C. psorophorae* in *Culiseta inornata*. The fungus was first discovered in 1956 in the irrigated districts of southern Alberta⁵. It has reappeared every year since that date and has often induced high mortalities in the mosquito populations. Attempts to establish infected colonies in the laboratory encountered the same problems noted by earlier workers. Limited infection was obtained by the Couch method, but only after adding water, algae and other substrates from the Canadian collection site. Efforts to increase the infection rate by modifying first the environment (for example, pH, temperature, water character, light and redox potential), second

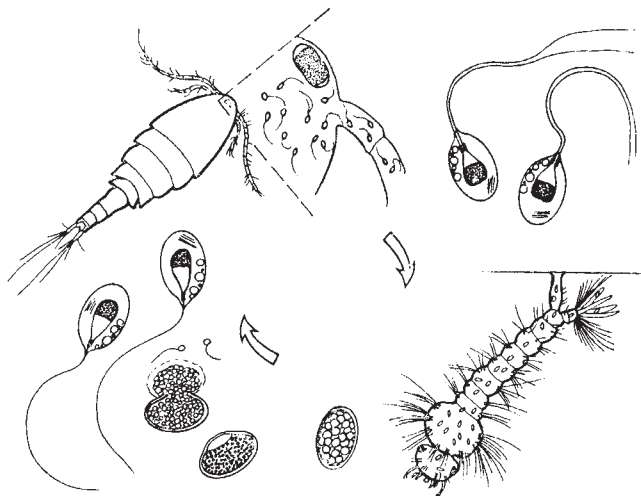


Fig. 1 Life history of *Coelomomyces psorophorae*. Resistant sporangia from the body of the mosquito release zoospores which infect *Cyclops*. Biflagellate spores from the *Cyclops*/*Callimastix* phase infect mosquito larvae.

the number or condition of the host and, third, the quantity of fungal inoculum, were inconclusive. This work did permit the removal of algae from the infection trials but did not materially improve their reliability.

Attempts to infect mosquitoes in simple, clean systems using synthetic pond water, and clean fungal inoculum failed repeatedly. Sequential addition of individual components from our 'gross' infection pans to our 'clean' trials has provided the key to the infection problem. Addition of the copepod *Cyclops vernalis* to pans containing fungi and mosquito larvae resulted in the appearance of infected mosquitoes. Control pans lacking copepods have never yielded diseased larvae, in spite of the addition of masses of zoospores and/or sporangia. This implied that *Coelomomyces* had an alternate, copepod host. To test this hypothesis, washed copepods, from pans with a record of high infectivity, were combined with mosquito larvae in synthetic pond water. Within a few days, 40% of these larvae were obviously infected with *C. psorophorae*.

Examination of the copepods revealed the presence of an 'unknown' phase in the life history of *Coelomomyces*. The body cavities of the diseased *Cyclops* were packed with swarming zoospores. At escape, the spores were found to be predominantly biflagellate, but otherwise similar to the zoospores produced in the resistant sporangia of *Coelomomyces*. Mosquitoes exposed to a suspension of these spores displayed typical *Coelomomyces* hyphal bodies and sporangia within a few days of inoculation.

These results indicate that the life history of *C. psorophorae* includes two different hosts (Fig. 1). Attempts to infect mosquitoes with spores from the resistant sporangia produced in the mosquito or to infect copepods with spores from other infected *Cyclops* have consistently failed and it seems that alternation of different hosts is obligatory. Such heteroecism is common in rust fungi, but has not been previously reported for the Chytridiomycetes or other phylumycetes.

The copepod phase of the fungus resembles the *Cyclops* parasite *Callimastix cyclopis* Weissenberg⁹. Originally described as a protozoan parasite, it has more recently been allied to the Blastocladales^{10,11}. The zoospores are characteristically multiflagellate, with all the zoospores from one copepod bearing the same number of flagella (for example, 2-5 per spore). The zoospore of *Callimastix* carries a peculiar paracrystalline body¹¹, which is also found in *Coelomomyces*^{12,13}, but has not been reported from other fungal zoospores. The ultrastructure of the unwallled plasmodia and spores, the swarming of the spores in the host

body, and the general morphological development of the copepod phase of *Coelomomyces* indicates that this phase may be considered as a biflagellate species of *Callimastix*. As the latter genus was described earlier than *Coelomomyces*¹⁴ it would seem to have nomenclatural priority.

The discovery of heteroecism in *Coelomomyces* has obvious implications for its possible use in biological control of mosquitoes. Whether other species and strains of *Coelomomyces* and *Callimastix* have complementary hosts requires further study. It is interesting to note, however, that in the earlier *Callimastix* studies, infected *Cyclops* were obtained from field collections or gross cultures and Vavra and Joyon¹¹ speculate on an unknown phase in the development of their copepod parasite.

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Absciscic acid levels in relation to drought tolerance in varieties of *Zea mays* L.

THE level of growth inhibitor in plants increases when they are subjected to water stress and the major constituent of this inhibitor is absciscic acid (ABA)¹⁻³. By its effect in closing stomata, ABA can control the rate of transpiration⁴⁻⁷ and it is therefore likely that ABA may be involved, to some extent, in the mechanism conferring tolerance of plants to drought.

Palacios⁸ and Muñoz⁹ have developed a line of maize (Latente) which shows enhanced ability to withstand drought conditions. Seed of this line was obtained from Mexico and it was decided to investigate whether its higher resistance to water stress was related to a capacity to produce appreciable quantities of absciscic acid. Accordingly we have compared the levels of ABA in detached leaves of Latente with those of two European varieties (Anjon 210 and LG 11) which have not been bred for tolerance to drought.

Seeds of each variety of *Zea mays* L. were sown in vermiculite and the seedlings raised in a growth room with a

Table 1 Determination of free ABA in detached leaves of wilted and non-wilted maize seedlings

	Free ABA (μg equivalents)					
	Latente		Anjon 210		LG 11	
	Control	Wilted	Control	Wilted	Control	Wilted
Experiment 1	12.8	50.3	2.4	21.1	3.1	11.8
Experiment 2	13.5	50.2	2.2	16.4	2.5	26.0
Mean	13.1	50.2	2.3	18.7	2.8	18.9

Data are from two independent experiments in which a Mexican line (Latente) and two European varieties of maize (Anjon 210 and LG 11) were grown in vermiculite.

15 h photoperiod (Mazda 'daylight' fluorescent tubes) at 21.5° C and a minimum relative humidity of 75%. At the end of the dark period on day 12, by which time ligules from the second leaves were exposed, 25 g batches of leaves were collected by excision 2 mm above the coleoptiles and laid on sheets of dry filter paper in a glass container. Warm air (35±2° C at leaf surface) from three hair driers was directed across the leaves until a loss of 10% in fresh weight had occurred (about 10 min). The leaves were then placed for 4 h in a closed glass chamber on four thicknesses of filter paper moistened with 20 ml distilled water. The controls were not subjected to the water stress treatment but were kept at the same high relative humidity for 4 h. At the end of this period, treated and control leaves were cut into small pieces and extracted in methanol for 48 h at -20° C. After the removal of the solvent under reduced pressure, the extract was partitioned twice between 2% sodium bicarbonate and redistilled ether. The aqueous fraction was washed with more ether and then acidified to pH 2.8 with 2 N H₂SO₄. The free acidic compounds were extracted with ether and the extract dried over anhydrous sodium sulphate. Most of the solvent was then removed.

Further separation was carried out on 20×20 cm fluorescent silica-gel coated TLC plates (Merck GF₂₅₄, 0.25 mm thick), which were developed in *n*-propanol-*n*-butanol-0.88 ammonium hydroxide-water (6:2:1:2 v/v) (ref. 10). The zone on each plate corresponding to an ABA marker spot (ultraviolet identification) was scraped from the chromatogram and eluted with methanol. Each eluate was concentrated for the second TLC stage and developed six successive times in chloroform-benzene-acetic acid (100:100:1.5 v/v). The silica in the ABA region was then removed and eluted with methanol. Final purification was carried out by ascending paper chromatography (Whatman No. 1) using isopropanol-0.88 ammonium hydroxide, water (8:1:1 v/v) and that part of the paper corresponding to the ABA zone (R_F 4.5-8.5) was divided into eight equal segments. These were assessed for inhibitory activity in the wheat coleoptile straight growth test¹¹. The mean length of the ten coleoptile sections used in each assay was expressed as a percentage of the length of the controls. A dose-response curve was obtained for synthetic (±) ABA so that the inhibitor extracted from maize could be expressed as μg equivalents of ABA (ref. 3).

The free ABA in detached non-wilted maize leaves in Latente, which is tolerant to water stress, was found to be more than four times that found in the less tolerant varieties Anjon 210 and LG 11. On subjecting the detached leaves to water stress the level of ABA increased in all varieties, confirming similar observations by Wright¹ and Wright and Hiron² (Table 1).

Although water stress produced a greater relative increase of ABA in the varieties Anjon 210 and LG 11 than in Latente, the level of ABA in the wilted Latente leaves was much higher. Such high levels would be expected to have a profound effect in closing leaf stomata thereby reducing transpiration and protecting the plant against water loss. Not only this, but the well-established effects of ABA in

inhibiting the activity of growth hormones would be expected to operate strongly, thereby restricting growth and conserving energy normally utilised in growth processes. Our results indicate that such protective mechanisms may be more well developed in the drought tolerant Latente maize than in the other two varieties studied.

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Altered fucolipid patterns in cultured human cancer cells

STUDIES in this laboratory¹ have shown that fucolipid metabolism is markedly altered in membranes of cells transformed by oncornaviruses. Transformed cells exhibit a sharp decrease in radioactive fucose incorporation into chromatographically less mobile, presumably more complex, fucolipids with a corresponding rise in radioactivity in more mobile fucolipids. Studies of fucolipid metabolism in normal rat kidney (NRK) cells transformed by a cold-sensitive transformation mutant of murine sarcoma virus (NRK (MSV-1b)) revealed a correlation between the appearance of the transformed phenotype and altered fucolipid synthesis². The fucolipids of SV40 and herpes simplex virus-transformed cell lines have also been examined³; some of the transformed lines were oncogenic while others were not when injected into appropriate animals. The cell lines which were capable of producing tumours had a block in the synthesis of the complex fucolipids comparable with that observed in the oncornavirus-transformed cells. In contrast, the transformed cell lines that did not produce tumours showed only a minor block in the formation of the complex fucolipids.

We have now examined the fucolipids of a number of cultured human tumour cell lines⁴. The results indicate a block in synthesis of the complex fucolipids that is comparable with that observed in virus-transformed cells.

The human tumour cell lines used included those isolated and characterised by Giard *et al.*⁴: A-375, malignant melanoma; A-204, rhabdomyosarcoma; and A-549 lung carcinoma. In studies⁴ with mice treated with antithymocyte serum (ATS), A-375 produced rapidly growing subcutaneous

tumours resembling amelanotic melanomas; A-204, slowly growing sarcomas; and A-549, rapidly growing subcutaneous and intracerebral carcinomas. Additional cell lines included: SV40-transformed human cell line (SV80)⁵ and 8387, a human fibrosarcoma cell line⁶. In addition, several strains of normal human embryonic cells were examined. The cells were grown in glass bottles in Eagle's minimal essential medium (MEM) supplemented with 10% foetal calf serum, and were labelled 3–5 d after plating with 1-¹⁴C-fucose (0.5 μ Ci ml⁻¹, 57 mCi mmol⁻¹) or with 1-³H-fucose (5 μ Ci ml⁻¹, 1.4 Ci mmol⁻¹). Cell monolayers were washed three times with cold phosphate-buffered saline (PBS), removed by scraping into PBS and sedimented to a pellet. The pellet was washed with PBS and the lipid was extracted¹.

Chromatographic analysis of the fucosylglycolipids was accomplished by thin-layer chromatography (Q-5 plates, Quantum Industries, Fairfield, New Jersey) in 2-propanol: NH₄OH:H₂O (7:2:1, by volume). The amount of radioactivity was estimated by scraping the plate in 1-cm strips from origin to solvent front and counting the fractions in a scintillation spectrometer¹. When ¹⁴C-fucose-labelled lipid was examined, the plates were visualised, before scraping, by exposure to Kodak No-screen X-ray film.

It seemed unlikely that nonlipid contaminants (that is fucose, GDP-fucose, fucose-1-phosphate or fucosepeptide) might have been present in the lipid extract. The materials characterised as fucolipids did not dialyse, were unaffected by snake venom phosphodiesterase or by *Escherichia coli* alkaline phosphatase, and did not have the same *R_F* values as those of authentic fucose, GDP-fucose or fucose-1-phosphate. These properties argue against the possibility of contamination with the small molecular weight material indicated above. Further, chromatography of the cytosol fucose radioactivity did not reveal components with the same *R_F* values as those of the fucolipids, whereas the lipid extract from a thoroughly washed high speed pellet yielded the identical fucolipid patterns as those obtained from

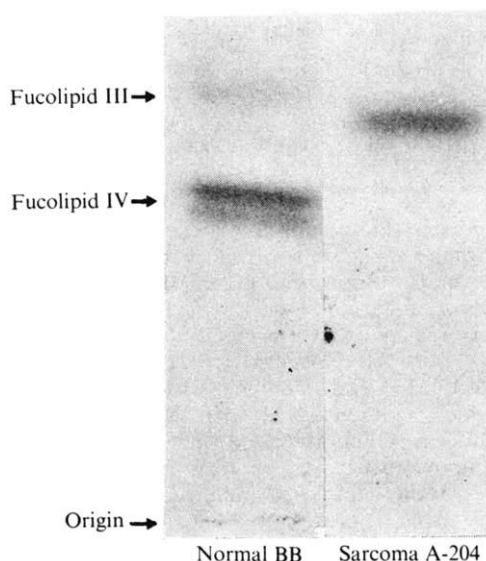


Fig. 1 Autoradiogram of one-dimensional chromatograms of ¹⁴C-fucose-labelled lipids of BB (normal human embryonic lung) and A-204 (sarcoma) cells. Cells were cultured in medium supplemented with ¹⁴C-fucose as described in the text, as are the conditions for collecting, extraction and chromatography of the fucolipids. The figure is a composite of autoradiograms of two thin-layer plates, 5 × 20 cm, chromatographed in parallel; solvent was CHCl₃:CH₃OH: NH₄OH (7:2:1, by volume). Fucolipids III and IV are shown; the more chromatographically mobile fucolipids (I and II) are not visible because of the relatively low level of radioactivity (see Table 1) in these components.

Table 1 Incorporation of ^3H -labelled fucose into lipids of normal human embryonic lung (BB) and human tumour cell lines

Fucolipid	Cell line											
	BB (normal)*			8387			A-204			SV80		
	%	c.p.m. per mg protein	%	c.p.m. per mg protein	%	c.p.m. per mg protein	%	c.p.m. per mg protein	%	c.p.m. per mg protein	%	c.p.m. per mg protein
I	2.8	166	6.8	494	6.7	394	1.8	167	10.3	506	16.3	852
II	8.3	491	11.0	799	10.7	630	3.6	333	14.7	722	24.5	1,281
III	16.7	987	21.9	1,591	80.0	4,711	73.2	6,772	50.0	2,457	44.9	2,347
IV	72.2	4,269	60.3	4,381	2.7	159	21.4	1,980	25.0	1,228	14.3	748

Each cell line was grown for 72 to 96 h (to obtain low and high cell density) in the presence of ^3H -fucose ($5 \mu\text{Ci ml}^{-1}$, 1.4 Ci mmol^{-1}). Conditions for collecting and for lipid extraction are indicated in the text.

* The fucolipid pattern of many different strains of normal human embryonic cell lines were studied, with comparable results to those seen for BB cells.

extracted whole cells. In addition, the fucolipids did not bind to DEAE-cellulose, which not only rules out negatively charged small molecular weight material (that is fucose-1-phosphate, GDP-fucose) but also makes it less likely that the compounds were fucoprotein. Further evidence to rule out the possibility of fucoprotein (or fucopeptide) included total resistance to exhaustive pronase digestion. It is also noteworthy that the Sephadex G-50 elution profiles of the major fucolipids (that is III and IV below) failed to manifest any overlapping with fucopeptides prepared according to Buck *et al.*¹⁰. We feel that the above evidence strongly supports the lipid nature of the fucose-labelled components. Nevertheless, we are attempting to accumulate a sufficient amount of these lipids, which are present in extremely low amounts, to carry out definitive chemical analyses.

Figure 1 is an autoradiogram of a one-dimensional chromatogram of the ^{14}C -fucose-labelled lipids of normal human embryonic cells (Fig. 1, lane 1) and of cultured human rhabdomyosarcoma (A-204) cells (Fig. 1, lane 2). Approximately equivalent amounts of radioactivity were chromatographed with each sample. It is apparent that the least chromatographically mobile fucolipid (that is FucCer IV) is greatly reduced in the rhabdomyosarcoma culture. In a comparable experiment the alternative isotopic form of fucose (^3H) was used to label the fucolipids of a number of human tumour cell lines, including those in Fig. 1. The results (Table 1) show that four of the tumour cell lines had a significant reduction in the incorporation of ^3H -fucose into fucolipid IV, the chromatographically least mobile component. In contrast, its incorporation into fucolipid IV in the 8387 cell line obtained from a fibrosarcoma was similar to that for the normal cell line. The experiment presented in Table 1 is representative of three separate experiments, each done in duplicate. In addition, comparable results to those seen in Table 1 were observed when ^{14}C -fucose was used with the same cell lines (Fig. 1). Several other normal human embryonic cell strains gave patterns similar to those shown for BB cells.

It has been shown that the membranes of human adenocarcinomas lack the blood group haptens A and B but accumulate the Le^a and H haptens⁷. More recently, Stellner and Hakomori⁸ demonstrated that the altered fucosylglycolipid patterns (that is blood group determinants) in adenocarcinoma tissue are due to a deficiency in glycosyltransferases rather than to enhanced hydrolase activity. Likewise, Fishman *et al.*⁹ noted the absence of a specific ganglioside galactosyltransferase in mouse cells transformed by murine sarcoma virus.

One of the problems in examining human tumour material is the difficulty of obtaining adequate normal control cells. Although we have not resolved this issue, it is noteworthy that regardless of the tissue source (kidney, lung or embryo) of the normal cells studied, or the cell type (epithelial or fibroblastic), or the species of primate from which the cells were derived, the fucolipid pattern was constant.

In the present study, altered patterns of incorporation of isotopically labelled fucose into the fucolipids of several

types of cultured human tumour cells were observed. The altered fucolipid patterns probably reflect changes in the glycosyltransferase activities similar to those reported⁸ for adenocarcinoma tissue. It is noteworthy that the fucolipid changes observed in the human tumour cells were similar to those observed in oncornavirus-transformed cells and in DNA-transformed, oncogenic cells. Three of the human tumour cell lines (A-204, A-549 and A-375) had been found to produce tumours in ATS-treated mice⁴. The cells of the 4th line, 8387, were fibroblastic in nature, although they piled up more than fibroblasts derived from healthy tissue; however, they failed to grow in ATS-treated mice⁶. In this manner, the 8387 line acted like those SV40- and herpesvirus-induced cell lines that are transformed but not oncogenic³. The SV80 line produced tumours in ATS-treated mice but they were then rejected, perhaps because SV40-transformed cells contain stronger antigens than spontaneous tumours⁵.

These studies on human cancer cells in culture are built on our previous investigations of virus-transformed lines. They offer a much needed biochemical method for identifying human cell lines that remain cancerous after being grown in culture.

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Essential fatty acids in cystic fibrosis

THE nature of the metabolic defect in cystic fibrosis (CF) has, to date, not been elucidated. Elliott¹ reported that intravenous administration of soybean oil and lecithin to children suffering from CF caused their sweat sodium concentration to decrease towards normal values. This prompted us to investigate the fatty acid spectra of the various serum lipid classes of children with CF. We have found that affected children are deficient in certain essential fatty acids.

in essential fatty acids may be produced by a reduced ability to absorb these acids from the diet and this, in turn, may result in defects in membrane structure or stability. Reduced levels of vitamin E in CF may reflect a reduced requirement for this vitamin because of the lower levels of essential fatty acids. It is also possible that the observed deficiency in serum levels of essential fatty acids may lead to less than normal production of prostaglandins. These approaches are under investigation.

This investigation was supported, in part, by funds from the National Cystic Fibrosis Research Foundation (to M.L.R.)

Table 1 Fatty acid spectra of serum lipid classes of children with cystic fibrosis and of normal children

Fatty acid	Cholesteryl ester		Triglyceride		Phospholipid	
	CF*	N†	CF	N	CF	N
12:0	Trace	Trace	Trace	Trace	Trace	Trace
14:0	1.3±0.07	0.9±0.02	1.9±0.22	1.8±0.20	Trace	Trace
16:0	25.3±0.95	22.6±1.30	40.9±1.00	38.1±0.33‡	43.1±0.80	43.6±1.71
16:1	7.0±0.89	2.7±0.43¶	5.0±0.39	2.0±0.13	Trace	Trace
18:0	7.5±0.94	4.6±0.73‡	7.4±0.48	15.4±2.00¶	25.1±0.58	21.7±1.44
18:1	32.2±1.34	27.1±0.81§	41.4±1.01	37.2±0.94§	20.5±1.13	14.0±0.95
18:2	23.3±3.22	38.6±2.88¶	3.5±0.32	5.3±0.97	6.4±0.32	13.0±0.69
20:4	3.2±0.33	3.4±0.90	—	—	4.9±0.36	7.7±0.68¶

CF, cystic fibrosis; N, normal. Figures give percentages of fatty acid present ± s.e.

* Five cases (four female, one male).

† Four cases (three female, one male).

‡ $P = < 0.05$.

§ $P = < 0.02$.

¶ $P = < 0.01$.

|| $P = < 0.001$.

Sera were obtained from five children (aged 4.5–12.5 yr) with CF, and compared with sera from four normal children (aged 5–12 yr). Serum lipids were extracted with chloroform-methanol (2:1)² and subjected to thin-layer chromatography on silica gel G using hexane-ether-glacial acetic acid (first 60:40:1 and then 90:10:1) as developing solvents³. The lipid classes were visualised on the plates with iodine vapour⁴ and after sublimation of the iodine, the appropriate areas were scraped, and the lipids were eluted and transesterified with a BF₃-methanol mixture. The resulting fatty acid methyl esters were analysed by gas-liquid chromatography using a column of 16% diethylene glycol succinate on 100–120 mesh Gas-chrom P. An argon ionisation detector was used. The column was standardised for retention times and quantitated at 175°C and 20 lb inch⁻² of argon gas using purified standards.

The results are summarised in Table 1. There are significant differences between CF and normal sera in all the classes of serum lipids analysed. In the cholesterol esters, the CF sera contained significantly more myristic, palmitoleic stearic and oleic acids and significantly less linoleic acid. In the triglycerides the CF sera were richer in palmitic, palmitoleic and oleic acids but contained less stearic acid. Serum triglycerides of CF children contained 34% less linoleic acid but the difference was not statistically significant. In the phospholipids, the CF sera contained significantly less linoleic and arachidonic acids and more oleic acid. Kuo and Huang⁵ found significantly less stearic and linoleic acids in the serum free fatty acids of CF children and significantly more palmitoleic acid and acids of chain length below that of palmitic acid (presumably myristic acid). Caren and Corbo⁶ found that the major change in the free fatty acid fraction of CF children was in the large increase in palmitoleic acid. Our data confirm their other findings which were assessed from the standpoint of relating CF to impairment of liver function.

The data suggest another approach to the study of the metabolic defect in cystic fibrosis. There is an apparent deficiency in essential fatty acids, and a marked reduction in serum vitamin E levels has also been reported in CF⁷. These conditions might be partially corrected by intravenous injection, or possibly feeding of, lipids containing essential fatty acids with concomitant administration of vitamin E. The observed deficiency

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Alteration of fibroblast gene products *in vitro* from a subject with Werner's syndrome

CULTURED human fibroblasts have a limited replicative capacity¹ and provide an excellent model for the study of biological ageing¹⁻⁴. Recent studies have demonstrated that toward the end of their replicative lifespan, normal fibroblasts accumulate a significantly higher proportion of defective enzymes and simultaneously incorporate increased amounts of amino acid analogues^{5,6}. In addition, the RNA base analogue, 5-fluorouracil induces premature senescence of cultured cells which is preceded by the appearance of altered enzyme⁵. These results have been taken to suggest that the fidelity of protein synthesis decreases, possibly causally, during cellular ageing *in vitro*⁵⁻⁷.

Cultured fibroblasts derived from subjects with Werner's syndrome, a hereditary disease of accelerated ageing, show a markedly curtailed lifespan compared to normal age-matched controls^{3,8,9}. Accordingly, one might predict that such cells would show the earlier appearance of abnormal gene products, a higher proportion of abnormal gene products, or a combination of both. Since it is difficult to obtain cultures from Werner's subjects before the disorder is clinically full-blown, all strains so far examined have grown poorly from the outset and have lost their replicative capacity quite prematurely. Thus, although the first prediction is difficult to test, the second possibility can be analysed. Here we demonstrate that a strain of Werner's fibroblasts contains a high defective proportion in 3 different gene products, HL-A2 and HL-A9, two histocompatibility antigens on the cell surface and glucose-6-phosphate dehydrogenase (G-6PD), a cytoplasmic enzyme in the pentose shunt.

Cultures were derived in our laboratory following explantation of skin of the anterior forearm as previously described². The subject with Werner's syndrome (H.McG.) was a 56-yr-old female who had already been studied *in vivo*¹⁰ and *in vitro*³. This strain of Werner's fibroblasts again showed a reduced lifespan so that only five to six subcultures were possible at a 1:8 split ratio, that is, 15–18 mean population doublings (MPD) before total cessation of growth^{1–3}. Normal controls were capable of

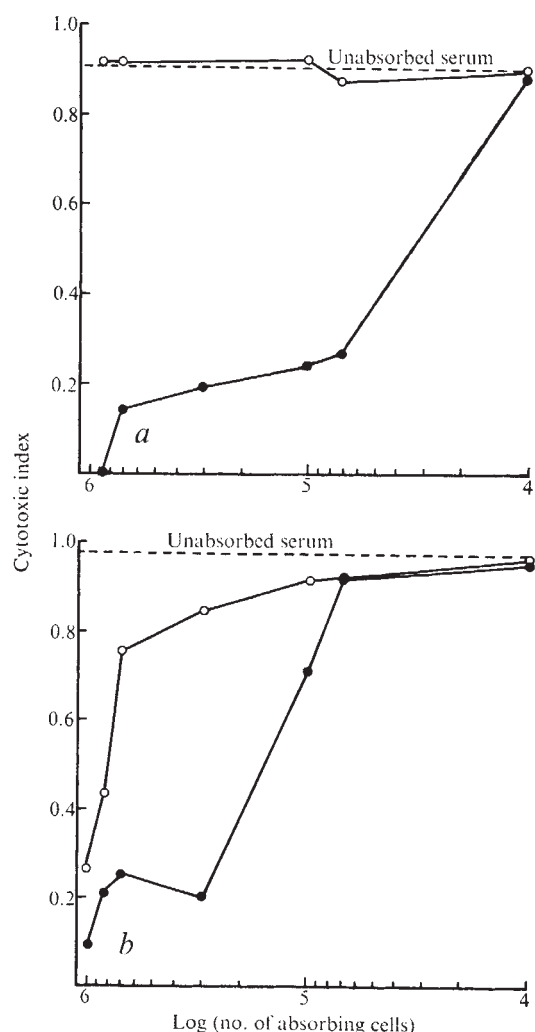


Fig. 1 Cytotoxicity of *a* anti-HL-A2 serum and, *b* HL-A9 serum following absorption by fibroblasts from Werner's syndrome and a normal 36-y-old male (HL-A2,9,7,12). Fibroblasts at the concentrations indicated were suspended in 10 μ l of antiserum and incubated for 40 min with mixing followed by centrifugation and testing of the supernatant against appropriate target lymphocytes. Werner's cells were at 15 and 12 MPD (total capacity 18) in *a* and *b*, respectively. Normal cells were at 21 and 36 MPD (total capacity 60). $\circ$, Werner's syndrome; $\bullet$, normal.

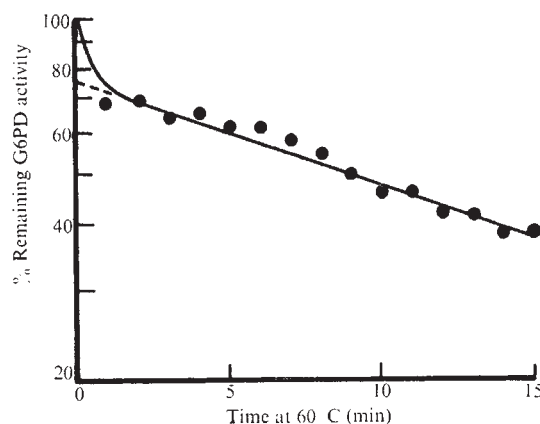


Fig. 2 Heat inactivation of glucose-6-phosphate dehydrogenase in extracts of skin fibroblasts derived from a patient with Werner's syndrome. Cells were at 12 MPD.

15–25 subcultures (45–75 MPD) in the same conditions. G-6PD was assayed for thermolability using crude cell extracts as described by Holliday and Tarrant⁸. Briefly, cultures were allowed to grow to confluence and then collected. Following rinsing, cells were suspended in 0.05 M Tris buffer, pH 8.0, containing 10^{-3} M NADP and sonicated followed by centrifugation for 30 min at 30,000g. The supernatant was then heated at 60°C. Aliquots were removed after various times and immediately placed in an ice bath till assay on a Gilford ultraviolet spectrophotometer. HL-A phenotypes were first determined on circulating lymphocytes of the skin fibroblast donors by a microdroplet cytotoxicity technique¹¹, modified slightly for fibroblasts¹². Quantitative absorption studies were performed using appropriate antisera as previously described¹³.

The HL-A phenotype of the patient with Werner's syndrome, as determined on her peripheral blood lymphocytes, was HL-A2, 9, 5, W15. All four antigens were detected on cultured fibroblasts by the cytotoxicity method but the results became erratic following the first subculture. The results of quantitative absorption studies for two antigens, HL-A2 and HL-A9, are given in Fig. 1. The expression of HL-A2 (Fig. 1*a*) was markedly reduced on fibroblasts from Werner's syndrome compared to the normal control. HL-A9 expression was also reduced (Fig. 1*b*) but not as markedly as that of HL-A2. Taking the 50% level of cytotoxicity as a reference point, the quantitative expression of antigens can be calculated^{13,14}. Thus, HL-A2 expression was reduced more than 50-fold in Werner's fibroblasts compared to the normal control while HL-A9 expression was reduced about 4.5-fold. The expression of each antigen in normal strains from donors aged 27–76 yr varies less than twofold throughout the culture lifespan as we and others had described previously^{15,16}.

Figure 2 indicates that extracts of Werner's fibroblasts had 24% heat-labile G-6PD. On two other occasions at similar MPD levels, Werner's cells had 14% and 22% heat-labile G-6PD. In contrast, early-passage cultures from normal controls aged 28–76 yr averaged 1.0% heat-labile G-6PD (range 0–4% in five strains tested 17 times) while late-passage controls averaged 4.3% (range 0–10% in four strains tested 17 times). An equal part mixture of extracts from Werner's cells (24% heat-labile) and normal cells (0% heat-labile) gave 9% heat-labile G-6PD, close to the predicted value of 12%. This suggests that proteolytic or other detrimental factors are not responsible for the greater degree of heat-labile G-6PD in Werner's cells and conversely, that a deficiency of stabilising factors is probably not involved.

The data indicate that three diverse gene products, HL-A2 and HL-A9, two surface-related antigens which are coded for by autosomal genes¹⁷, and G-6PD, a cytoplasmic enzyme which is genetically X linked¹⁸, are markedly abnormal in cultured cells from Werner's syndrome. Virtually identical

results on G-6PD have recently been found on a different strain of Werner's fibroblasts by Holliday *et al.*⁹, although the differences between early and late-passage normal cells in this report are much less than previously observed by Holliday and Tarrant in MRC-5, a strain of male foetal lung fibroblasts⁵. We recognise that controversy exists regarding the composition of HL-A antigens¹⁹⁻²¹. Indeed, if these molecules turn out to be glycoproteins²² it is possible that their expression depends on glycosyl transferases as well as the protein component coded for by HL-A genes. Thus, the altered reactivity with antiserum of HL-A2 and HLA-9 in Werner's fibroblasts may reflect aberrations in more than a single gene product. But this does not affect the conclusions and it seems likely, therefore, that these cells have a widespread defect in cellular proteins which is more severe in extent than in ageing normal cultures. In other studies, we have found similar disturbances in HL-A antigen expression^{13,15} and heat lability of three different cytoplasmic enzymes in fibroblasts derived from subjects with progeria, another disease of premature ageing (S.G., unpublished). Additionally, a fraction of clonal subpopulations within normal mass cultures may also develop altered HL-A expression during ageing *in vitro*^{12,15}.

Although no proof exists that accumulation of defective proteins is the cause rather than the result of cellular ageing⁷, our evidence strengthens the concept that the rate of fibroblast ageing *in vitro* is directly related to the rate of ageing *in vivo*. Nevertheless, information is still needed to explain how a diversity of protein defects in ageing cells predisposes individuals with Werner's syndrome to diabetes mellitus, severe atherosclerosis and neoplasia¹⁰, the same diseases that increase exponentially during normal biological ageing^{4,15,23,24}.

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Effects of prostaglandin E₂ on human platelet adenyl cyclase and aggregation

THE ability of prostaglandins to affect platelet function has been described in many laboratories^{1,2}. The most potent of these, prostaglandin E₁ (PGE₁), has been found to inhibit platelet aggregation and stimulate platelet adenyl cyclase. The effect of prostaglandin E₂ (PGE₂), however, has been either inhibitory or stimulatory, depending both upon the species of animal examined³ and the concentration of prostaglandin used⁴.

Recent demonstrations of the ability of platelets to produce and secrete prostaglandins^{5,6} have stimulated new interest in the effects of the different prostaglandins on platelet activity.

The present investigation was designed to re-examine the effect of PGE₂ on human platelets, using concentrations of prostaglandin comparable with the levels that platelets produce and secrete^{5,6}. It was found that PGE₂ stimulated platelet adenyl cyclase and inhibited both ADP- and collagen-induced platelet aggregation.

Blood was collected into sodium citrate (0.38%) from normal human donors who had not ingested aspirin for a minimum of 14 d. Blood or platelets contacted only siliconised glass or metal surfaces, or non-wettable plastic. The platelet-rich plasma (PRP) was collected after centrifugation at 150g and maintained at room temperature. Aggregation was followed by the turbidimetric procedure of Born⁷, measuring absorbance decrease at 600 nm at 37° C in either a Gilford 2000 or Unicam SP800 spectrophotometer with magnetic stirring (1,000 r.p.m.). Varying concentrations of either PGE₁ or PGE₂ (Ono Pharmaceuticals) dissolved in dimethylformamide (DMF) (10 µl) were added to the PRP (2.7 ml) and incubated without stirring at 37° C for 10 min. At time zero, inducers were added and stirring commenced. Appropriate controls preceded and followed each PG aggregation. Inducers were ADP (Sigma), 10 µl, in aqueous solution (final concentration, 0.9×10^{-4} to 1.8×10^{-4} M) and collagen, 50 µl, prepared according to Hirsch, *et al.*⁸.

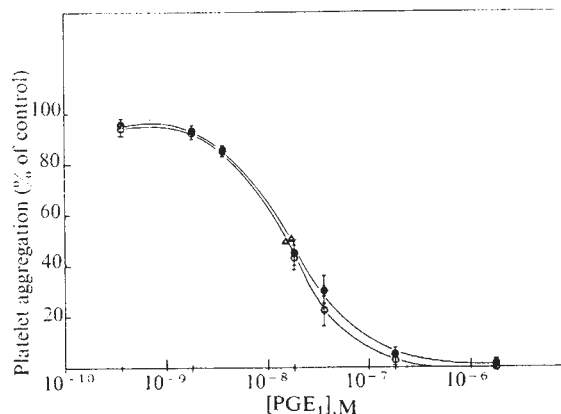


Fig. 1 The dose-dependent inhibition by PGE₁ of the aggregation of human platelets in citrated PRP, induced by ADP. The ordinate axis is expressed as percent of control values for both initial rate (●) and total absorbance change (○). Vertical bars show standard error of the mean (s.e.m.), 5-8 replications per point. The ID₅₀ concentration is marked on each curve (Δ). For other details, see text.

Adenyl cyclase studies utilised platelets obtained from EDTA-anticoagulated blood by the method described by Haslam⁹. The washed platelets, resuspended in 10 mM Tris (pH 7.5 at 20°C), were kept intact at 4°C until used. Cyclase activity was determined in triplicate by measuring conversion of α - 32 P-ATP to 32 P-cyclic AMP. Added 3 H-cyclic AMP was used as an index of recovery. Platelets were used without being lysed. Lower levels of cyclic AMP were obtained in platelets lysed either by sonication or freeze-thawing.

The assay mixture contained the following components in a final volume of 1.0 ml: α - 32 P-ATP (New England Nuclear), 6 mM (0.5 μ Ci); phosphoenolpyruvate (Sigma), 0.1 mM; pyruvate kinase (Sigma), 1.0 unit; magnesium chloride, 9 mM; theophylline (Sigma), 10 mM; cyclic AMP (Sigma), 2.0 mmol; potassium chloride, 5.5 mM; Tris buffer, 40 mM, pH 7.5 (20°C); approximately 10^8 washed human platelets.

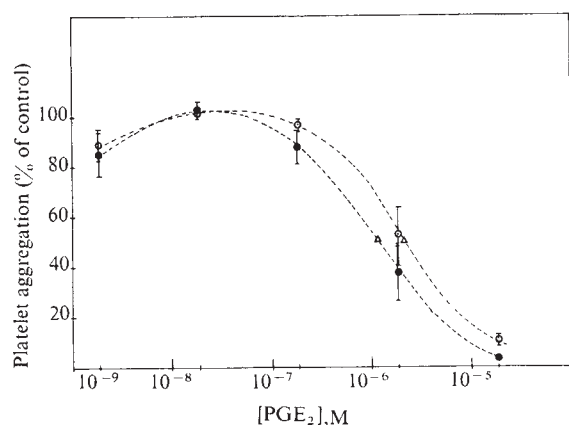


Fig. 2 The dose-dependent inhibition by PGE₂ of the aggregation of human platelets in citrated PRP, induced by ADP, 8–12 replications per point. The ordinate axis, symbols and details are as in Fig. 1.

As in the aggregation work, prostaglandins were added in DMF, with appropriate blanks and controls. Methodology used by one of us (M.D.) differed slightly and was described previously¹⁰.

After incubation at 37°C for 15 min, the reaction was stopped by adding 0.1 μ Ci of 3 H-cyclic AMP (50 μ l) to each tube followed by heating for 5 min in boiling water. The cyclic AMP isolated by the methods described by White¹¹ and Ramachandran¹² was counted for both 3 H and 32 P.

PGE₁ inhibited the ADP-induced aggregation of citrated PRP in the expected dose response relation over a concentration range of 3.7×10^{-10} to 1.8×10^{-6} M (Fig. 1). This was evident both when the inhibition was measured as decrease in initial absorbance rate and when it was measured as total absorbance change. The correlation between the two measured parameters was excellent with an ID₅₀ of 1.6×10^{-8} to 1.7×10^{-8} M.

The effect of PGE₂ on ADP-induced platelet aggregation in citrated PRP was similar to that of PGE₁. Thus, over a concentration range of 1.8×10^{-9} to 1.8×10^{-5} M, only inhibition was observed in both the initial rate (ID₅₀ = 5×10^{-6} M) and total absorbance change (ID₅₀ = 1×10^{-5} M) parameters (Fig. 2). PGE₂ was therefore only 1/500 to 1/250 as potent as PGE₁ in inhibiting the ADP-induced aggregation of citrated PRP. Stimulation by PGE₂ alone or augmentation of ADP-induced aggregation by PGE₂ was never seen.

The collagen-induced aggregation in the same system was also inhibited by PGE₁ (Fig. 3) over the same concentration range. ID₅₀, measured by initial rate inhibition, was 1.2×10^{-6} M. For total absorbance change, the ID₅₀ was 2.1×10^{-6} M. Although it seems that greater inhibition was obtained at the lowest PGE₂ concentration, than at a tenfold higher concentration, these data were not statistically different from control values.

Platelet adenyl cyclase was stimulated by both PGE₁ (5.0×10^{-9} to 5.0×10^{-6} M) and PGE₂ (1.0×10^{-7} to 2.0×10^{-4} M) as shown in Fig. 4. Both compounds displayed only stimulation above basal levels, even at the lowest concentrations, in classic dose-response relationships. The ED₅₀ for PGE₁ was 5×10^{-8} M and for PGE₂ was 7×10^{-6} M. Thus, about 140 times more PGE₂ was required to equal a given PGE₁ level of stimulation. Although absolute amounts of adenyl cyclase activity varied among platelet samples, the ED₅₀ values obtained for both PGE₁ and PGE₂ were consistent and reproducible. These effects were obtained whether the platelets were prepared as described here or by a previously published method¹³.

Several studies using PGF_{2 α} (Fig. 4) were also performed. Though stimulation was minimal, a decrease of adenyl cyclase activity below basal levels was never seen.

In the platelet aggregation studies, the effects of PGE₁ and PGE₂ were inhibitory only, as measured by two parameters over a wide concentration range. Contrary to published results^{3,14}, no stimulation of either ADP- or collagen-induced aggregation at low PGE₂ concentrations was observed. The difference between this and previous studies may be in the reproducibility of aggregation results. In citrated PRP, even a series of control experiments on the same platelet sample will yield results that vary with time and other factors we have not yet identified, under presumably identical conditions. To circumvent this, the values obtained for each prostaglandin treatment were compared with control values obtained both before and after the PGE inhibition experiment. Many experiments were performed at each inhibition concentration with many different platelet samples. In the ADP-induced aggregation studies inhibited by PGE₂ below 2×10^{-6} M, over 60 separate experiments, exclusive of controls were performed with platelets from 17 different donors. The collagen-induced experiments were replicated fewer times, hence the larger standard errors (Fig. 3). Even in these experiments though, inhibition was obvious.

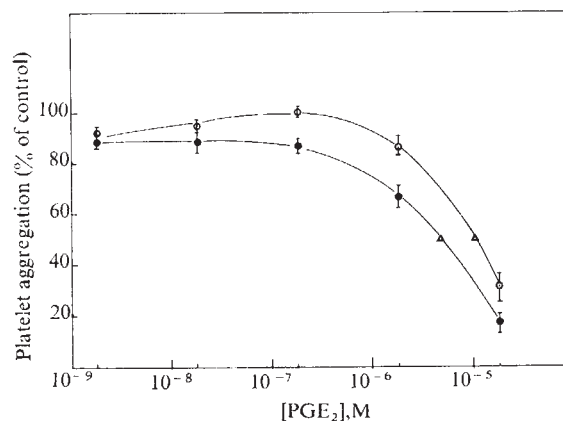


Fig. 3 The dose-dependent inhibition by PGE₂ of the aggregation of human platelets in citrated PRP, induced by collagen, 4 replications per point. The ordinate axis, symbols and details are as in Fig. 1.

The stimulation of platelet adenyl cyclase activity by PGE₁ at various concentrations agrees with previously reported data^{10,13}. PGE₂ similarly elicited no response other than stimulation of the platelet adenyl cyclase. This is contrary to previous published reports⁴ in which PGE₂ at 2.8×10^{-7} M (0.1 μ g ml⁻¹) was found to inhibit adenyl cyclase activity. In the earlier work, emphasis was given to the finding that only at low concentrations did PGE₂ inhibit adenyl cyclase activity. Concentrations which we found stimulatory, however, were the same as those previously reported as inhibitory. The reason for this discrepancy is not clear.

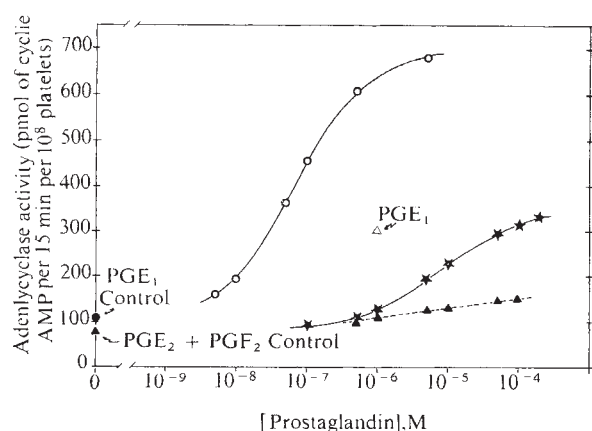


Fig. 4 The effects of PGE₁ (○), PGE₂ (★), and PGF_{2α} (▲) on washed human platelet adenylyl cyclase. The PGE₁ data were obtained using different platelets from the PGE₂ and PGF_{2α} data. For reference, a single concentration of PGE₁ (△) was assayed in the PGE₂-PGF_{2α} platelets. In other experiments not reported, PGE₂ and PGF_{2α} were studied at concentrations as low as 10⁻⁸ M with results similar to PGE₁ (M.D.). At PGE₁ concentrations above 10⁻⁵ M, a decreased output of cyclic AMP has been observed (unpublished data). It has not been possible to show this effect with PGE₂, possibly because of difficulties in getting high enough concentrations in solution.

Recent studies have suggested the existence of a regulatory or feedback mechanism of platelet function in which prostaglandins, produced and secreted in response to thrombin, may stimulate platelet adenylyl cyclase to produce cyclic AMP and thereby modulate the release and aggregation processes (refs 6, 15 and M.D., unpublished data). Our findings that both PGE₂ and PGE₁ stimulate adenylyl cyclase supports this hypothesis, especially as we found that presumably physiological levels of PGE₂ (refs 5, 6) were stimulatory.

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Delayed fertilisation in senescent golden hamsters

REPRODUCTIVE senescence in the female golden hamster occurs after 14 months of age and is characterised by a significant decline in the production of offspring¹. Recent studies in our laboratory concerned with the cause(s) of this decline have revealed large numbers of non-viable ova in aged hamsters before implantation² and a developmental delay of approximately 12 h in preimplantation and implantation stages of embryos from senescent hamsters when compared with young females at the same hour of pregnancy^{3,4}. Since the elapsed time for the union of gametes is important for normal fertilisation and development we examined ova for the onset of fertilisation in young and aged hamsters.

One hundred and twenty young virgin (3-5 months old) and 170 multiparous senescent (14-17 months old) hamsters, cycling regularly, were mated with young males (3-6 months old) at the onset of behavioural oestrus. Ovulation was considered to occur 8 h from the onset of oestrus⁵ and all developmental stages are based upon this assumption. All animals were from the Oregon colony, except for 72 senescent females from commercial breeders. Ova collected from 1 h through 12 h and at 1, 1½, 2, 2½, 3 and 3½ d intervals, were either mounted *in toto* and examined with differential interference contrast microscopy (Nomarski optics) or compressed, fixed and stained with lacmoid.

Most of the ova from senescent females showed a 2 to 5 h delay in fertilisation compared with young females (Table 1). The delay was not merely an inbred characteristic of females from the Oregon colony, since aged females from two commercial animal farms demonstrated the same trait.

Although there seemed to be a short delay in the completion of ovulation in the senescent hamster 1 h after ovulation (10.5±1.12 compared with 13.8±0.91 for young females), there was no statistical difference in the number of ova at 2 h (12.8±2.25) compared with ova from young hamsters 1 h after ovulation. The total number of ova recovered from young and senescent females was similar throughout the first 12 h (young 13.2±0.33, senescent 12.1±0.38), but a real difference was apparent when the number of normal-appearing ova was determined (young 12.6±0.40, senescent 10.9±0.30); large numbers of non-viable ova were found in the aged hamsters. Some ova were apparently defective at the onset of ovulation since they were in an advanced state of deterioration at the time fertilisation normally occurs in young females. Other fertile ova of normal appearance stained with lacmoid revealed anomalies commonly associated with a delay in fertilisation, such as fragmented pronuclei (6 ova), dispermy (3 ova), trispermy (1 ovum) and androgenesis (1 ovum). On examining zygotes

Table 1 Normal ova and their stage of fertilisation from young and senescent hamsters

Hours after ovulation	Ova penetrated by spermatozoa		Percentage Ova with second polar body		Ova with second* polar body and male and female pronuclei	
	Y	S	Y	S	Y	S
2	—	0.0	—	0.0	—	0.0
3	44.2	16.7	29.5	8.3	0.0	0.0
4	90.1	37.3	77.5	25.3	0.0	0.0
5	100	40.9	94.3	29.6	75.0	1.6
6	—	51.5	—	36.6	—	20.9
7	—	78.0	—	65.9	—	41.5
8	100	94.4	100	78.9	100	73.7
9	—	80.3	—	78.9	—	78.9
10	100	72.3	100	69.1	100	69.1
11	84.5	94.1	84.5	91.2	84.5	91.2
12	96.1	100	96.1	98.4	95.4	91.1

Y, Young.

S, Senescent.

* Ova stained with lacmoid.

from the uterus, we found that approximately 40% of the ova from senescent females were incapable of implantation so that preimplantation loss seems a major factor. Unfertilised ova made up a small percentage of the non-viable ova (1.4% compared with 1.3% for young females); therefore, it is conceivable that the delay in fertilisation was sufficient to have caused some embryonic loss.

Researchers have shown that delaying fertilisation in young golden hamsters by artificially inseminating⁶ or naturally mating females⁷ 3 h after ovulation, results in considerable embryonic loss (14% to 61%). In both studies capacitation of the spermatozoa would have required between 2 and 4 h (ref. 8); therefore, the ova were between 5 and 7 h old before fertilisation. In our study many ova from aged hamsters were not fertilised until 5–8 h after ovulation.

Spermatozoa may be slowed in their ascent through the aged reproductive tract, although oviducts compared from several young and senescent females allowed to breed for 1 h from the beginning of coitus revealed no difference in the approximate number or location of spermatozoa. No spermatozoa were detectable, however, in ampullae of oviducts of either group. A more probable cause for the delay is prolonged capacitation. This may explain why some ova from senescent hamsters in the present study are fertilised at a time when hamster spermatozoa have been shown to be no longer viable⁹. Raised levels of plasma progesterone have recently been reported in some senescent hamsters during oestrus and the first day of pregnancy¹⁰. This may indicate that the ageing hamster uterus is less capable of binding progesterone, a condition noted *in vitro* with the senescent rabbit uterus¹¹. A refractory uterus could be causing a delay in fertilisation in hamsters since subcontraceptive levels of progesterone may delay fertilisation in rabbits¹² either by slowing spermatozoa migration through the reproductive tract and/or by prolonging capacitation^{13,14}.

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Abnormal potassium conductance associated with genetic muscular dystrophy

DYSTROPHIC diseases not only lead to progressive deterioration of muscle, but alter several properties of the erythrocyte surface^{1–4}, and permeability of various other membranes^{5–7}. Muscular dystrophies are also associated with defects in the nervous system, so that a neurogenic aetiology⁸ has been

suggested, there being reports of peripheral neural dysfunction⁹, increased frequency of mental retardation¹⁰ and defective auditory acuity¹¹.

Many of these non-muscular (systemic) features of the dystrophic condition are associated with cellular membranes; indeed dystrophic muscle itself has an abnormal membrane composition¹² and greater than normal calcium-linked ATPase activity in the sarcoplasmic reticulum¹³. Moreover, dystrophic muscle exhibits a significantly altered ionic content and abnormally large potassium conductance¹⁴, although these attributes may only reflect the characteristic degeneration of such tissue. I report here that diverse membrane systems in dystrophic animals and humans conduct potassium more rapidly than in the case of normal subjects. This higher conductance could, in principle, account for the disease process.

Table 1 shows passive K⁺ efflux from erythrocytes from normal subjects and patients with Duchenne muscular dystrophy where the rate of efflux is about five times greater. In other experiments, the cardiac glycoside, ouabain, was without influence on the efflux and addition of the K⁺-conducting antibiotic, valinomycin, stimulated it. The extent of K⁺ loss in the presence of valinomycin, suggests that there is no consistent difference between the K⁺ content in normal and dystrophic erythrocytes.

Table 1 Efflux of potassium from human erythrocytes

Source of cells	No. of subjects	Potassium efflux (nmol per min per 10 ⁸ cells)
Normal subjects	8	21.6 ± 2.4
Duchenne dystrophy patients	7	107.9 ± 7.5

Results are expressed as mean and standard error of the mean. Samples of venous blood were drawn into a heparinised tube, cells were sedimented at 1,500g, resuspended in isotonic saline, washed twice and finally resuspended in about one-tenth of the original volume of saline. All operations were carried out at 0°C. Potassium efflux was measured at 20°C with a Corning model 476132 potassium electrode which exhibited an 80-fold selectivity of potassium as compared with sodium. The medium contained 360 mM sucrose, 15 mM NaCl and 0.1 mM KCl in a volume of 10 ml to which was added 0.4 ml of an erythrocyte suspension containing approximately 10⁹ cells per ml.

Figure 1 shows light scattering changes after addition of potassium acetate to suspensions of mouse liver (a) and brain (b) mitochondria. Normal mitochondria from both organs are relatively impermeable to K⁺ whereas those from dystrophic animals took up a significant amount. I have observed a similar increase in K⁺ conductance with liver mitochondria from a second strain of dystrophic mice (129ReJ-dy) and from nutritionally dystrophic rats maintained on a vitamin E-deficient diet. This last observation suggests that membrane dysfunction may be the basis for similarity between genetic and nutritional dystrophy. Addition of Na⁺ and Li⁺ acetate does not result in significant changes in light scattering by mitochondria from either normal or dystrophic animals. In dystrophic mitochondria, the uptake of K⁺ is probably energy-linked, as subsequent addition of 2 μM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone, an uncoupler of oxidative phosphorylation, returns the absorbance to its initial value.

Thus there is greater than normal conductance of K⁺ across erythrocyte, liver mitochondrial and brain mitochondrial membranes in the dystrophic state. Since none of these tissues seem to degenerate as muscle does, the genetic defect in muscular dystrophy may give rise to membranes, throughout the organism, which are leaky to K⁺, and muscle deterioration may be a consequence of that leak. This may occur through defective excitation-coupling where rapid K⁺ movement might serve to collapse charge dislocation required for a cycle of membrane depolarisation–repolarisation. In this connection, it is interesting that the T tubular system, a major locus for K⁺ transport to the muscle interior, has been shown to be structurally altered in muscular dystrophy¹⁵.

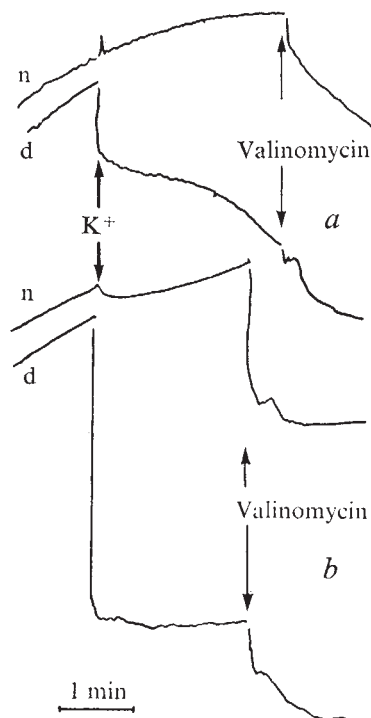


Fig. 1 Light scattering changes caused by addition of potassium acetate to mitochondria. *a*, Liver mitochondria; *b*, brain mitochondria. *n* denotes mitochondria isolated from normal mice (Bar Harbor strain C57BL/6J); *d* denotes mitochondria isolated from genetically dystrophic mice (Bar Harbor strain C57BL/6J-dy²J). K⁺ denotes addition of 4.0 μ eq potassium acetate; valinomycin denotes that of 0.5 μ g of the potassium ionophore, valinomycin. Light scattering was measured as the change in absorbance at 540 nm at 20°C, a downward deflection corresponding to swelling (uptake). Liver mitochondria were isolated in 250 mM sucrose while brain mitochondria were isolated in a medium containing 300 mM mannitol and 0.1 mM ethylenediamine tetra-acetate and adjusted to pH 7.4. The light scattering measurements were carried out in 3.0 ml of the appropriate isolation medium supplemented with 5.0 mM bicine, pH 7.4. The vertical bar in the figure indicates an absorbance difference of 0.003 units.

Finally, the K⁺ leak as an explanation for the origin of muscular dystrophy is consistent with the observation that hypokalaemic periodic paralysis, which clearly reflects anomalous K⁺ distribution across membranes, frequently occurs in families with a history of muscular dystrophy¹⁴. It should also be viewed in light of the report that serum creatine phosphokinase activity, a measure of muscle deterioration, decreases in dystrophic patients after administration of lithium gluconate¹⁶, which is a non-competitive inhibitor of K⁺ translocation in various biological systems¹⁷.

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Possible role of zonula occludens of the myelin sheath in demyelinating conditions

AUTOIMMUNE mechanisms may play a major role in several human demyelinating diseases¹. Myelin contains components, principally a basic class of protein, which, when injected systemically with an adjuvant, elicit in the experimental animal a demyelinating encephalomyelitis or neuritis with perivascular infiltration of lymphocytes and inflammatory cells^{1,2}. A satisfactory hypothesis is that the potential self-antigens are secluded from the immune systems in the normal living animal³. Evidence for such a specific compartmentation of myelin components, however, has been lacking up to now. This has led several authors to suggest alternative explanations^{4,5}. Here we demonstrate the structural basis for a seclusion mechanism within both peripheral and central myelin sheaths in different animal species.

A continuous tight junction, or zonula occludens, is present at the borders of each myelin segment. This junction separates an intramyelinic extracellular space from the intercellular compartment of the nervous tissue. In addition, the junctional strand separates the internal portion of the glial membrane exposed to the tissue space from the internal portion of the glial membrane exposed to the intramyelinic extracellular space and constituting the compact myelin.

Blocks of peripheral nerve and of various regions of the CNS, including cerebellum, medulla oblongata, optic nerve and forebrain, were obtained from turtles, chickens and rats perfused with fixatives containing aldehydes, or with ferrocyanide-reduced osmium tetroxide⁶, and processed for thin sectioning and freeze-fracturing according to routine methods^{7,8}. In a more extensive article⁹, we have shown that thin sectioning of blocks fixed with ferrocyanide-osmium and freeze-fracturing confirm the existence, already partly established in the literature¹⁰, of an extracellular space within the myelin sheath, not only in the peripheral but also in the central nervous system. This extracellular space is situated between the paired minor dense lines (that is, the external leaflets of the myelin membrane) as predicted from X-ray diffraction studies¹¹.

In sections, the tight junctions of the myelin sheath appear as spots of membrane fusion (Fig. 1 *a, b*). In peripheral sheaths they occur at the internal and external

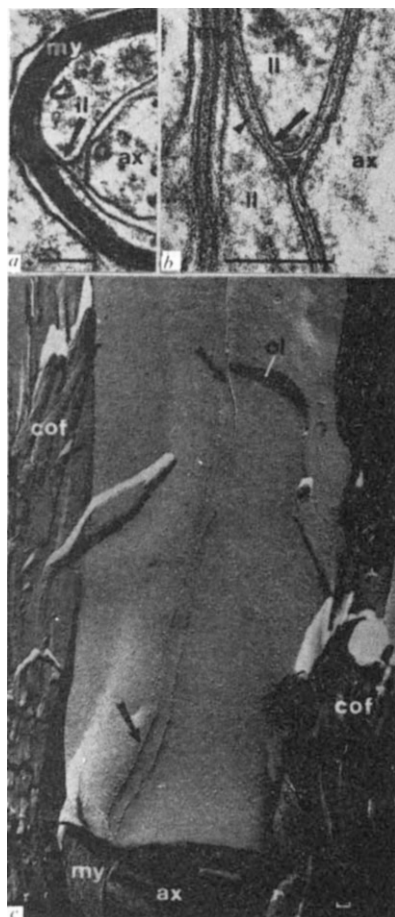


Fig. 1 *a*, Electronmicrograph of a central myelinated fibre showing the tight junction (arrow) of the internal mesaxon, that is, the site where the inner loop (il) joins the compact myelin (my). The axon is labelled ax. *b*, a central myelinated fibre showing the tight junction (arrow) between two paranodal loops (ll) near the Ranvier node. The paranodal loops represent the winding lateral loop passing repeatedly through the plane of section (compare Fig. 2 *d* and *e*). The arrowhead indicates the intramyelinic extracellular space, and the triangle the periaxonal space. *c*, Electronmicrograph of a replica of a freeze-fractured peripheral myelinated fibre. The arrows indicate tight junctional strands cleaved with the A face of the Schwann cell membrane at the external mesaxon (that is, the site where the outer loop terminates the myelin sheath). The cross-fractured outer loop is labelled ol; cof indicates collagen fibres. The scales in the photographs represent 0.1 μ m.

'mesaxons' and between adjoining 'paranodal loops' (terminology explained in the figure legends). In central sheaths the tight junctions occur at the mesaxon (internal), between adjoining paranodal loops³², and at the site where the outer loop (or outer tongue) joins the compact myelin. The latter site is considered, therefore, as the equivalent of the external mesaxon of peripheral sheaths.

Freeze-fracturing confirms these findings. This technique splits cell membranes, thus producing two artificial fracture surfaces, referred to as A (cytoplasmic) and B (external) 'faces', which complement each other in the hydrophobic interior of the membrane¹². As for the tight junctions in other tissues¹³, strands (Fig. 1 *c*) and furrows are seen on the A and B faces, respectively, at all sites where spots of membrane fusion were observed in thin sections. In addition, the replicas of freeze-fractured myelin sheaths give a more complete tridimensional picture of this tight junctional system, demonstrating that there is a continuous zonula occludens extending from the outer to the lateral and inner loops. This is shown schematically in Fig. 2 *d*, *e*, where the myelin sheaths have been represented as 'un-

wrapped'. The outer, lateral and inner loops represent portions of the continuous marginal belt of the glial sheet which contains a rim of cytoplasm.

Tight junctions are also present between the loops of the circumferential Schmidt-Lanterman incisures and between the longitudinal protoplasmic islands. These are channels of cytoplasmic retention which occur in some sheaths and which interrupt the compact myelin. The strand(s) of these junctions are continuous with the junctional strands of the mesaxons⁹. There is, therefore, no interruption of the zonula occludens at these sites.

It has been demonstrated in several organs that the zonula occludens, although partly permeable to water and small solutes, constitutes an efficient barrier to the extracellular diffusion of large molecules¹³. While the joining of three neighbouring cells in epithelia might represent the site of small leaks, the zonula occludens of the axonal sheath is formed by a single cell, and the barrier might turn out to be particularly effective. Lanthanum has been observed occasionally to penetrate into the myelin sheath^{13,14}, but this might be an artefact. Tracers of large molecular weight do not enter the intramyelinic extracellular space¹⁶⁻¹⁸. The existence of a zonula occludens was not considered in the experiments with tracers. We suggest here that it is the zonula occludens at the inner, outer and lateral borders of the sheets forming the myelin tube that makes the myelinic extracellular space a secluded compartment, thus sequestering some myelin components. According to various authors, the basic encephalitogenic protein is located, at least in

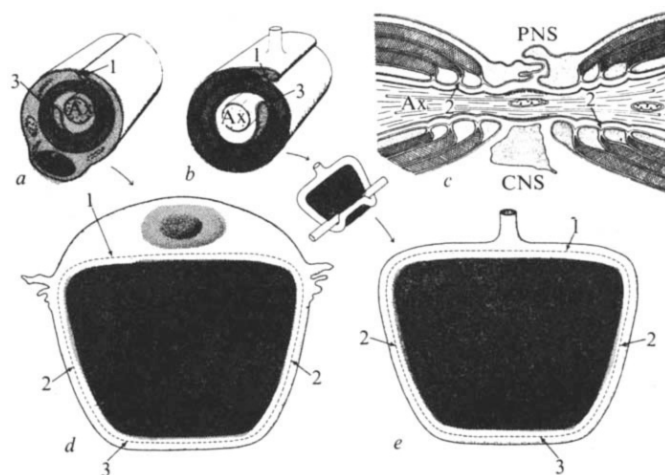


Fig. 2 Diagrammatic representation of peripheral and central myelin sheaths. The illustrations *a*, *b* and *c* are quasi-realistic. In *d* and *e* the axon (Ax) has not been represented, and the complete sheath of one internode has been figuratively unrolled as shown in the unlabelled inset. Portions of peripheral (*a*) and central (*b*) myelin sheaths in tridimensional view. The intramyelinic extracellular space (cross-hatching), comprised between the paired minor dense lines, is emphatically exaggerated. The arrows point to the tight junctions separating this space from the extracellular space within the tissue (arrow 1) and the periaxonal space (arrow 3) (also exaggerated). *c*, Schematic view of the Ranvier node and the paranodal region of peripheral (PNS) and central (CNS) myelinated fibres in longitudinal section. The arrows (labelled 2) point to tight junctions of the spirally winding lateral loop. The periaxonal space is communicating with the extracellular space through the channels of the paranodal axoglial junction. This junction, corresponding to the scalloped portion of the axolemma, has not been represented here and is described in refs 9, 31-33. Peripheral (*d*) and central (*e*) myelin sheaths represented as unwrapped. Arrows 1, 2 and 3 indicate the outer, lateral and inner cytoplasmic loops, respectively. The dashed line shows the extent of the zonula occludens encircling the area of compact myelin (shaded area). For the sake of simplicity, this dashed line corresponds to only one-half of the complete junctional specialisation and is drawn on the middle rather than on the side of the lateral loops and on the axonal aspect of the inner loop rather than on the opposite aspect.

part, at the minor dense line^{19,20}, that is, it borders on the intramyelinic extracellular space. Following damage to the myelinic tight junctions, components of this normally secluded compartment may leak out, thus provoking phagocytic and ultimately autoimmune reactions.

The presumptive role of the zonula occludens suggested above is probably a corollary of other functions which might be: first, the maintenance of the homeostasis of the extracellular material providing interlamellar adhesion in the compact myelin; and, second, the separation of two different intramembranous regions of the same cell, that is, the glial outer membrane and the membrane of compact myelin.

There are experimental conditions in which degeneration of the myelin sheath and its removal by phagocytes is produced without concomitant damage to the axon²¹⁻²⁵ but without proven involvement of the immune systems. We wonder whether or not the effect of the experimental damage involves the strand(s) of the zonula occludens also in these cases.

There are other organs and tissues which contain potential self-antigens. We are particularly struck by certain analogies between the spermatogenic system and the myelin. Sperm contain antigens which can elicit formation of serum sperm agglutinins and immobilisins as well as cellular infiltrates in the testis^{26,27}. It has been shown²⁸ that in the spermatogenic pathways there is a luminal barrier to extracellular diffusion constituted by epithelial zonulae occludentes. In addition, at the seminiferous tubules, where sperm are formed, there is a blood-testis barrier²⁹. Thus, the sperm are secluded from the immune systems³⁰. The compartmentation produced by the myelinic zonula occludens and by the blood-brain barrier¹⁶ might have, to some extent, functional implications analogous to the compartmentation in the spermatogenic system.

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Light-evoked release of glycine from the retina

GLYCINE is one of the amino acids now regarded as putative neurotransmitters. It is present in high concentration in the retina^{1,2} and an active high affinity uptake system into a type of amacrine cells has been demonstrated^{3,4}. Moreover, glycine has inhibitory effects in the retina^{5,6}.

It has been demonstrated in other parts of the central nervous system that amino acids can be released by electrical stimulation or ionic shock⁷⁻¹⁴. It has also been shown, however, that such crude methods of stimulation will release glycine (and some other amino acids) from tissues where it is unlikely to be a neurotransmitter¹⁵. We have therefore studied the release of glycine from the retina, which can easily and effectively be stimulated by light flashes, either *in vivo* or *in vitro*.

The anterior segment and the vitreous were carefully removed from the eyes of Nembutal anaesthetised cats, exposing the retinae for the *in vivo* studies. Rabbit retinae were carefully excised with the choroid and maintained in a small organ bath at 37°C for the *in vitro* experiments. The retinae were exposed to tritiated glycine by superfusing them at a rate of 1 ml min⁻¹ for 10 min with a salt solution containing 40 µCi glycine-2-³H or by injecting 10 µCi intravitreally (rabbits) 2 h before excising the retina. Initially 0.9% NaCl was used in the cats; but later, Krebs-Ringer-bicarbonate was used with equal results. In the rabbit, the solution described by Ames¹⁶ was used. The retinae were washed (1 ml min⁻¹) in darkness with the salt solution for 20-40 min and then exposed to one or two 10 min periods of light flashes (2 H₂) 10 min apart. From the washing and onwards, the salt solution contained 0.1 mg ml⁻¹ glycine. The radioactivity of the superfusate was monitored by liquid scintillation spectrometry either directly or in the glycine spot in thin layer silica gel chromatography (solvent: *n*-propanol: water, 70:30 v/v).

The site of uptake of radioactivity into retinae was checked by autoradiography in two experiments and was found to be almost exclusively in a type of amacrine cells, in good agreement with previous results^{3,4,17}. More than 95% of the radioactivity

in the retinae or in the superfusate moved like glycine in the chromatograms.

Thirty-three experiments were performed on rabbit retinae. The radioactivity of the superfusates was usually decreasing throughout the experiments, on the average by a factor 0.79 (s.e.m. = ± 0.029) during the 5 min preceding the light stimulation. During this, however, the radioactivity increased by a factor 1.30 (s.e.m. = ± 0.103), measured from the start of the stimulation to the peak of the release (which appeared after 3–7 min). The difference between the two factors is highly significant ($P < 0.001$ in the Student's two-tailed t -test). One experiment is shown in Fig. 1. In this particular one and also in another, small aliquots of the superfusate fractions were chromatographed, and the radioactivity of the glycine spots were measured. A peak was seen, exactly matching that of the crude superfusate fractions (see Fig. 1). Autoradiograms of the thin layer chromatograms did not reveal any other radioactive spot.

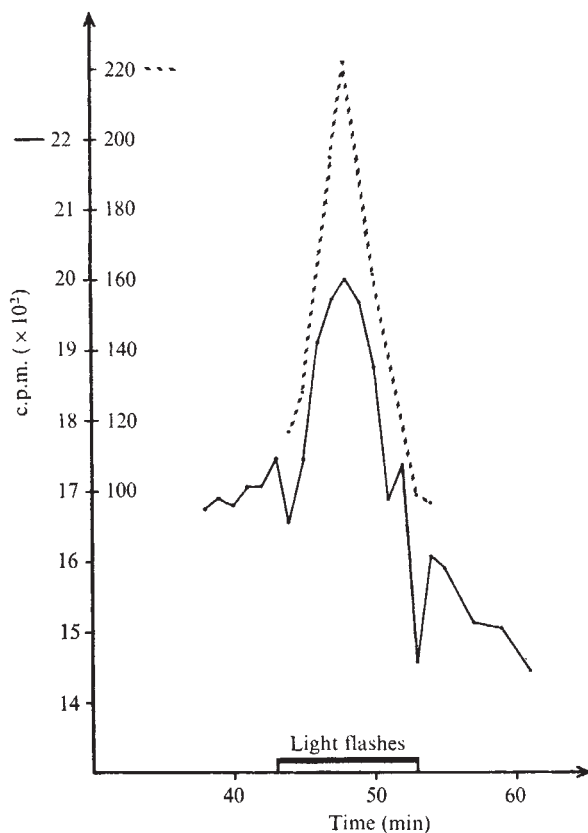


Fig. 1 Efflux of radioactivity in a single experiment from light stimulated rabbit retina *in vitro* after incubation with ^3H -glycine. —, radioactivity of the crude effluent (scale on the left hand side of the ordinate); - - -, radioactivity in the glycine spot from silica gel thin layer chromatograms of the effluent (scale on the right-hand side of the ordinate). The difference in absolute radioactivity in the two curves is caused chiefly by differences in sample size. The light flashes were given at 2 Hz with a xenon flash tube driven by 0.6 J per flash and located 30 cm from the retina.

The ^3H -glycine was given by intraocular injection in 14 of the 33 rabbit experiments, and by incubation in the remaining 19. There was no statistically significant difference between the results of these two subgroups. The increase in the outflow of radioactivity was highly significant ($P < 0.005$) in each of them.

A second stimulation was given in 13 of the rabbit experiments, and resulted in a smaller and less distinct increase of the radioactivity of the superfusate than the first stimulation.

During the five minutes immediately before the second stimulation the radioactivity on average decreased by a factor 0.92 (s.e.m. = ± 0.042), and during the stimulation it rose on average by a factor 1.15 (s.e.m. = ± 0.100) above the starting value. The difference is possibly significant ($P < 0.05$).

In the experiments on anaesthetised cats, the results were very variable, usually for obvious technical reasons such as incomplete removal of the vitreous, bleedings clogging the superfusion cannulas, movements of the animal and so on. In about twenty attempts, five superfusates showed a smooth baseline curve with a clear peak appearing during the light stimulation. On the average, the peak of these five curves reached 2.94 times (s.e.m. = ± 0.485) above the level at the beginning of the stimulation, this contrasts with the preceding 5 min, when the radioactivity fell, on average, by a factor of 0.79 (s.e.m. = ± 0.093).

Thus, light stimulation can release radioactivity from retinae preloaded with ^3H -glycine. The chromatograms of the superfusate or of the whole retina suggest it is glycine that is released. Serine is a precursor and also a metabolite of glycine, however, and does not separate well from it in chromatograms. Therefore, the experiments do not show to what extent the released radioactivity might represent serine. This is not highly neuroactive, however, and is not generally regarded as a putative neurotransmitter. On the other hand, glycine has been shown to fulfil several criteria of a neurotransmitter in the retina: it is present in high concentration^{1,2}—although the precise cellular location remains to be established; there is an efficient neuronal uptake mechanism that conceivably inactivates released glycine^{3,4}, and applied glycine has clear inhibitory effects^{5,6}.

Our demonstration of light-induced release of radioactivity from retinae preloaded with radioactive glycine further supports that it is a retinal neurotransmitter. It is of particular importance that the release can be achieved both *in vivo* and *in vitro* and with a physiological stimulus, as this suggests that it is the result of normal nerve activity.

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Does the central human retina stretch during accommodation?

CLEAR evidence that the human retina stretches during accommodation was presented by Moses¹, but his measurements were all made at the anterior margin of the retina, so they did not show how this stretch is spatially distributed. If it is largely confined to the region of the *ora serrata*, its contribution to the perceptual correlates of accommodation will be much less than if the central retina is distorted as well. A perceptual effect involving accommodation-dependent distortions in the visual field has recently been attributed by its discoverers² to such a stretching of the central retina, although non-retinal explanations for this effect are also tenable.

It seemed worthwhile to do an experiment in which the possibility that the central human retina stretches during accommodation could be evaluated, and in which the magnitude of any stretch which was found could be assessed.

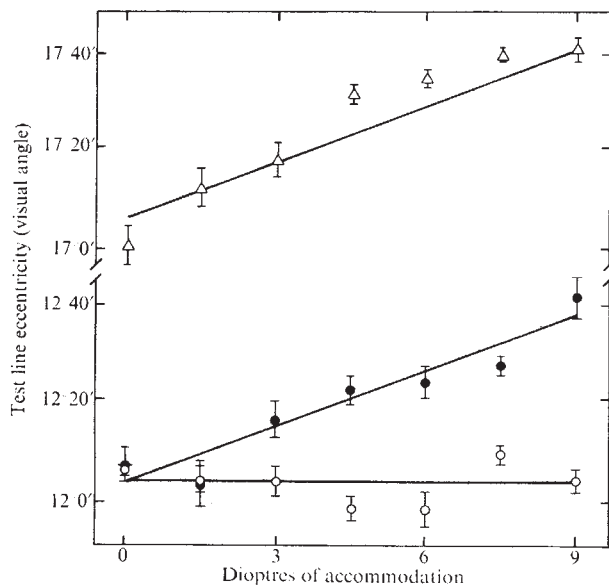


Fig. 1 Test line settings made by the first observer are shown as a function of dioptres of accommodation. Closed filled circles refer to the near edge of the blind spot, the line drawn through them by eye indicating a 4.7% increase in visual angle over the range of accommodation. A line parallel to this has been drawn through the triangles which refer to the far edge of the blind spot. The open circles are control data obtained during cycloplegia: a horizontal line has been drawn through them to indicate that there is no stretching of the retina in these conditions. Error bars show 1 s.e.m. above and below each point, which is the mean of ten determinations. A long test line (2° from top to bottom) and a high retinal illuminance for the background (5.6 log trolands) were used to make the line as conspicuous as possible once it had emerged from the blind spot.

The observer's left eye was patched throughout, and the right provided with a Maxwellian view of a fixation cross and a vertical test line, both seen in silhouette against a background of monochromatic yellow (575 nm) light. The test line was located to the right of the fixation cross; its exact horizontal position was under the observer's control. Because the eye was positioned one focal length behind the Maxwellian lens, the apparatus served as a Badal optometer: that is, movements of the target plane (containing both the fixation cross and the test line) along the optic axis stimulated different degrees of accommodation but did not affect the size of the retinal image.

The observer's task was to fixate the centre of the cross and to keep it in sharp focus, while at the same time moving the test line until it was just visible on the near side of the blind spot (the side closer to the fixation cross). Measurements were

made at seven different levels of accommodation (Fig. 1) to plot the distance in visual angle between the fixation cross and the test line. Since the test line marks the near edge of the blind spot, the data imply that the fovea and the optic disk are more widely separated when accommodation is strained than when it is relaxed.

An alternative interpretation of the data would be that threshold criteria changed systematically as a result of accommodative effort, or that the target was focused on the retina more sharply at one level of accommodation than at another. These possibilities were tested, as a class, by measurements analogous to the first set but now localising the far side of the blind spot: if blurring or criterion shifts were involved, the line should have been set closer to the fixation cross as accommodation increased. Just the opposite was found (Fig. 1), thus eliminating these possibilities. The results of these two experiments were confirmed on a second observer.

There remained the chance that some misalignment of the apparatus, rather than a stretching of the retina, produced the effect. To rule out this possibility a control was run with accommodation paralysed with 1% Cyclogyl, but with the target plane moved along the optic axis as before. A smaller aperture stop was now used to reduce the diameter of the pencil of light passing through the pupil centre to less than 0.5 mm, thereby ensuring that the stimuli would always be seen in sharp focus despite the lack of accommodation. The effect found earlier was now clearly absent (Fig. 1).

A final experiment was undertaken to determine how great a contribution to the effect is made by changes in the magnifying or other properties of the crystalline lens. It is known³ that convergence can bring about accommodation; and it seems likely that in a presbyope, devoid of accommodative ability, convergence would still be accompanied by a central command for accommodation. The lens would not respond to such a command, but if the ciliary muscle contracted, then the retina might be stretched just as in a younger subject.

This possibility was investigated using a 66-yr-old presbyopic observer. A different apparatus (with slightly different stimulus dimensions) was employed so that the observer could be presented with the fixation cross binocularly, while the test line was as usual seen by the right eye only. Natural viewing at a distance of 3 m was used, and the observer moved the test line with a pulley so that it fell on the near margin of the blind spot. While the right eye remained in the primary position throughout, mirrors were introduced in front of the left eye to make it converge a total of 25°. Measurements made both with and without the mirrors showed that the visual angle between the fixation cross and the blind spot increased some 4.6% when convergence was brought into play; this is comparable to the figures obtained during actual accommodation in the initial experiments: 4.7% for one subject (age 28) and 4.4% for the other (age 23). This similarity of results for the presbyopic and non-presbyopic observers suggests that changes in the lens do not play a primary role in producing the perceptual effect under study.

The results of the present study, taken together, argue persuasively that the central region of the human retina stretches substantially—some 4½%—during marked (9 dioptre) accommodation. This is much larger than the figure of 2% stretch, which is obtained by dividing Moses' measured 0.5 mm advance of the *ora serrata* by the distance from the *ora* to the optic disk⁴; the reason for this difference remains unknown. The stretching reported here has implications for our understanding of accommodative micropsia⁵ and other perceptual phenomena: in particular the stretch (if it is non-uniform) may underlie the spatial distortions discovered by Blank and Enoch², as in fact they propose, but this remains to be established. The stretching of the central human retina may also be of interest to those who study the eye from a clinical point of view.

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Acetylcholine as an excitatory neuromuscular transmitter in the stomatogastric system of the lobster

THERE is much physiological evidence that L-glutamate acts as an excitatory transmitter at many arthropod neuromuscular synapses^{1–3}. The only suggestion that acetylcholine (ACh) has such a function is due to Futamachi⁴. He showed that the nerve terminal region innervated by the largest crayfish slow flexor excitatory motor neurone is depolarised by iontophoretic applications of ACh, but not L-glutamate. I now have biochemical and physiological evidence that ACh is the neurotransmitter at some, but not all, of the excitatory neuromuscular synapses of the lobster stomatogastric system.

The stomatogastric ganglion of the spiny lobster, *Panulirus interruptus*, contains about 30 neurones, 23 of which are motor neurones and make excitatory neuromuscular synapses with 38 pairs of striated muscles which move the lobster's stomach⁵. These muscles are typical of crustacea, with fibres approximately 100 μm in diameter and up to several centimetres long. According to present knowledge, their sole motor innervation is from the stomatogastric

ganglion⁵. The motor neurone somata are 40–100 μm in diameter, show both attenuated action potentials and considerable sub-threshold synaptic activity⁶, and are identified physiologically by the motor nerve in which their axons are found⁷.

Choline acetyltransferase catalyses the enzymatic biosynthesis of ACh and correlates well with the presence of ACh in *Aplysia* neurones⁸. I have assayed this enzyme in individual somata of physiologically identified stomatogastric neurones. First, neurone somata were penetrated with microelectrodes for identification of the cells, a drawing was made of their relative positions, and then the cells were dissected individually into small glass tubes by the method of Giller and Schwartz⁹, after being frozen in a mixture of 70% ethylene glycol and 30% *Panulirus* saline. The tubes containing the cells were kept frozen on dry ice until the assay was performed, usually within 2 d of dissection.

Each ganglion contains 2 PD (pyloric dilator) motor neurones and one LP (lateral pyloric) motor neurone⁶. Table 1 shows a summary of choline acetyltransferase assays on single PD and LP somata. The PD somata contained choline acetyltransferase activity in 16 out of 18 cells assayed. The actual values obtained showed a large variation which may reflect damage caused during the identification or dissection, or actual variation in the amount of the enzyme present in different cells. The mean of these measurements is 12.6 ± 8.1 pmol ACh per cell per h which may be somewhat low because I made no attempt to discard values from cells which may have been damaged. I estimate that the amount of enzyme is about 125 pmol ACh produced per nl cell volume per hour. This value is comparable with those found in cholinergic neurones in *Aplysia*⁹.

Table 1 Acetylcholine transferase in single PD and LP somata

	pmol ACh, per cell per h*	No. of somata with activity No. of somata assayed
PD cells	12.6 ± 8.1	16/18
LP cells	$<2.7 \pm 0.8$	1/10
Limit of sensitivity	2.4 ± 0.7	

* Means plus standard deviations of values from 10 experiments.

PD cells showed activity in all but two cells assayed, with a range between 4.0 and 29.9 pmol ACh per cell per hour. The LP cells were below the limit of sensitivity in all cases but one, where a low level of activity was found. Assays were performed as by Coggeshall *et al.*¹⁰. 10 μl of buffer-substrate (final concentrations, 0.1 M NaPO_4 , pH 7.9; 0.25 M NaCl; 10 mM choline chloride; 1 mM EDTA; 0.1 mM eserine; 16 mM MgCl_2 and 0.4 mM ^{14}C acetyl CoA, specific activity 5–10 $\mu\text{Ci } \mu\text{mol}^{-1}$ (New England Nuclear Corp.)) was added to the tubes containing cell somata and to empty tubes (blanks). Tubes were incubated at 38°C for 30 min. The reaction was stopped with 100 μl of 50 mg ml^{-1} sodium tetraphenylboron in 3-heptanone. Tubes were mixed, 100 μl of 0.2 M NaPO_4 , pH 7.0, was added, mixed and spun in a table-top centrifuge for 2.5 min. 50 μl of the organic phase was counted in 10 ml of scintillation fluid. Ten to twenty blanks were run with each assay, and the limit of sensitivity was estimated on the basis of the variation in the blanks and the specific radioactivity of the reaction mixture.

In all experiments but one, the LP cell showed no acetylcholine transferase activity. As the LP cell is frequently the largest cell in the ganglion, and is always one of the largest, this difference in enzyme activity is not a trivial consequence of differences in the size of the PD cells and LP cell within a ganglion.

The PD neurones innervate the dorsal dilator muscle (muscle cpv la,b according to Maynard and Dando's terminology⁹). Figure 1 shows the effects of bath application of ACh and L-glutamate on the dorsal dilator muscle. Figure 1a shows the tension produced by 2×10^{-5} M ACh with 0.01 mg ml^{-1} Tensilon (an acetylcholinesterase inhibitor). Dose-response curves show that the threshold for

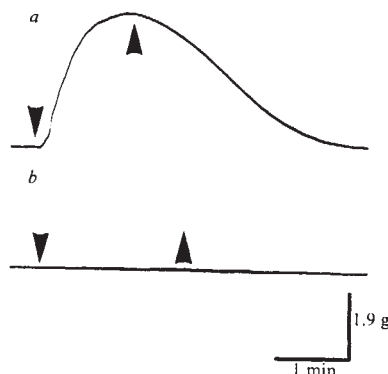


Fig. 1 Effects of bath application of ACh and L-glutamate on a dorsal dilator muscle. The muscle was connected to a Grass FT03 force displacement (tension) transducer, and the output was displayed on an oscilloscope and photographed with a kymograph camera. The muscle was placed in a 1.5 ml chamber through which saline flowed continuously at 6–8 ml min^{-1} . a, 2×10^{-5} M ACh with 0.01 mg ml^{-1} Tensilon was introduced into the chamber at the downward arrow. The muscle contracted, and the upward arrow indicates the start of the wash in normal saline. b, 1×10^{-3} M L-glutamate was introduced to the chamber at the downward arrow, causing no response, and the wash was started at the upward arrow. The muscle continued to contract in the presence of ACh for 6 h after the test with L-glutamate.

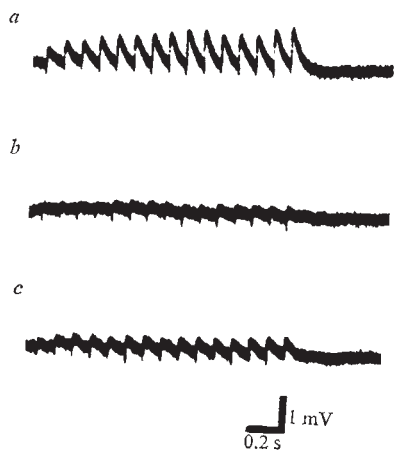


Fig. 2 The ejps recorded in a dorsal dilator muscle fibre are reversibly blocked by D-tubocurare. The ejps were recorded with a 30 M Ω 2.5M KCl-filled electrode and conventional electrophysiological equipment. The ejps were elicited by stimulating the PD motor nerve with a suction electrode in trains of 15 pulses at 10 per s for 1.5 s. *a*, ejps recorded in normal saline; *b*, the same penetration 7 min after the addition of 5×10^{-5} M D-tubocurare. The ejps were almost completely blocked; *c*, the same penetration after 10 min wash with normal saline. The ejps returned, although not to the size in (*a*). The resting potential was -64 mV.

contraction was 1×10^{-6} M ACh and that maximal tension was produced by 2×10^{-5} M ACh. D-Tubocurare (1×10^{-4} M) shifted the ACh dose-response curve to the right, characteristic of a competitive inhibitor of the ACh response. 5×10^{-7} M α -bungarotoxin (gift from Dr Hannah Friedman) completely blocked the response to 2×10^{-5} M ACh. Figure 1*b* shows that 10^{-3} M L-glutamate produced no tension in this muscle, nor did high concentrations (10^{-4} M) of dopamine, serotonin, octopamine, L-aspartate, GABA, glycine, noradrenaline or taurine. In a similar experiment the muscle innervated by the LP neurone (p2a)⁹ did not contract when 1×10^{-3} M ACh with Tensilon was added, although when the nerve innervating the muscle was stimulated, the muscle did contract¹¹. These results show that ACh causes contraction in the dorsal dilator muscle while the LP muscle does not respond to ACh.

Figure 2 shows that excitatory junctional potentials (ejps) in the dorsal dilator muscle fibres were blocked reversibly by D-tubocurare, a known cholinergic antagonist. After 7 min in 5×10^{-5} M D-tubocurare, the normal ejps (Fig. 2*a*) were considerably attenuated (Fig. 2*b*); after 10 min of

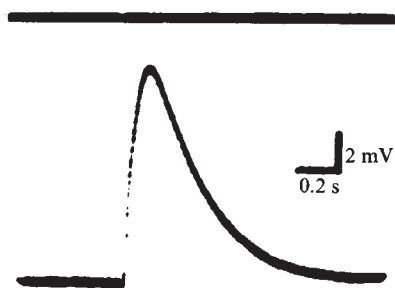


Fig. 3 Iontophoretic application of ACh to the surface of a dorsal dilator muscle fibre. The iontophoretic electrode was filled with 3 M ACh and had a resistance of about 90 M Ω . A bucking voltage was applied to minimise leakage of the ACh. A 30 M Ω electrode filled with 2.5 M KCl recorded membrane potential of the fibre. The top trace shows a current monitor. The iontophoretic pulse was 100 nA for 20 ms. The bottom trace shows the intracellular recording electrode. Resting potential was -65 mV. Horizontal calibration bar is 200 ms. Vertical calibration bar is 100 nA for the top trace and 2 mV for the bottom trace.

washing in normal saline they had recovered, although not completely.

Figure 3 illustrates the response of a dorsal dilator muscle fibre to iontophoresis of ACh on to its surface. Iontophoretic responses of this kind can be recorded from many areas of these fibres. The time course and amplitude of the potentials recorded are very sensitive to the position of the ACh electrode, and the ACh response desensitises with repeated applications to the same spot.

The data presented here suggest very strongly that ACh is the neurotransmitter at the PD neurone-dorsal dilator muscle synapse, but not at the LP neurone-LP muscle synapse. Definitive proof of this requires further experiments, but it is clear now that the PD and LP cells utilise different neurotransmitters. Furthermore, it is difficult to argue that L-glutamate is the neurotransmitter at the PD-dorsal dilator synapse, which clearly points to the dangers in making sweeping generalisations about 'the arthropod excitatory neuromuscular transmitter'. It is interesting that there is no known peripheral inhibition in the stomatogastric system¹², while many arthropod muscles receiving L-glutamate excitation also receive inhibitory innervation. It is also important that the PD and LP neurones make inhibitory synaptic connections within the stomatogastric ganglion⁶. There appears to be heterogeneity in excitatory neuromuscular transmitters among the neurones within this one crustacean ganglion, and some of these neurones appear to make cholinergic neuromuscular junctions.

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Methylation of ribosomal proteins *in vitro*

STARVATION of an *Escherichia coli rel⁻* (RC^{rel}) strain for an essential amino acid results in the synthesis of ribosomal RNA (rRNA) without the concomitant synthesis of proteins^{1,2}. Ribosomal precursor particles accumulate in these conditions and such precursor particles have been shown to be deficient in the methylation of rRNA (ref. 3). We have observed that in addition to proteins L7 and L12, which

were previously reported to be methylated⁴, several other ribosomal proteins from the 50S subunit were also methylated in the unstarved culture^{5,6}. Similar observations have also been made by Alix and Hayes⁷, but are the precursor particles generated by *rel⁻* strain after starvation for methionine also deficient in the methylation of the ribosomal proteins and if so, can such particles be methylated *in vitro*?

Here we have determined the proteins that were methylated *in vitro*. More than 80% of the incorporated methyl groups was found to be located in one protein (L11). The methylated amino acids present in this protein were also characterised. This information could enable the isolation and purification of protein methylases and the study of the function of protein methylations.

E. coli strain 1500, an *rel⁻* mutant of a K-12 strain isolated by Dürwald and Hoffmann-Berling⁸, was obtained from the Coli Genetic Stock Center in New Haven, Connecticut. The *met B* marker was introduced by P1 transduction from JC-355 which is a K-12 *met⁻* strain originating with A. J. Clark. Cells were grown with a limiting methionine concentration (0.8 $\mu\text{g ml}^{-1}$) and collected 1.5 h after exhaustion of methionine. During this period of starvation, RNA continues to be synthesised at a linear rate. About 1.2 g of cells were suspended in a buffer containing 0.05 M Tris-HCl, pH 7.8, 0.1 mM MgCl₂, 0.05 M KCl, and were disrupted with a French press. After centrifugation at 30,000 g for 30 min, the supernatant fluid was layered on top of a 5–20% linear sucrose gradient prepared in the same buffer and centrifuged overnight at 18,000 r.p.m. in an SW 25.2 rotor. Fractions containing the 50S peaks were collected and concentrated by ethanol precipitation⁹. Analysis of RNA by the gel electrophoresis procedure of Dahlberg *et al.*¹⁰ revealed that 20% of the total RNA in the pooled 50S particles was in the precursor form. The analysis also showed the presence of less than 4% 16S rRNA which was due to the contamination by 30S subunits. The methylating enzymes were obtained from the 0.5 M KCl wash of 70S ribosomes according to the procedure of Vogel *et al.*¹¹ from cells grown in the presence of 20 $\mu\text{g ml}^{-1}$ methionine.

Figure 1 shows that the methylation of the 50S ribosomal proteins, as analysed by the one-dimensional polyacrylamide gel electrophoresis, was dependent upon the addition of the methylating enzymes. In the absence of the added enzyme (Fig. 1a), very little methylation occurred and the

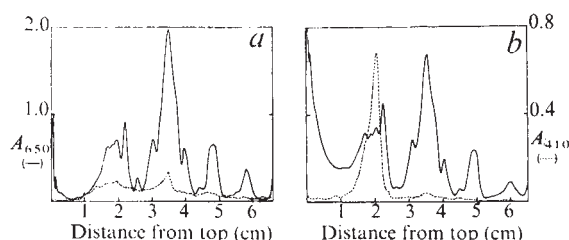


Fig. 1 Identification of the proteins that were methylated *in vitro* by one-dimensional polyacrylamide gel electrophoresis. The *in vitro* methylation of *E. coli* 50S particle was carried out in a final volume of 0.15 ml containing 0.05 M Tris-HCl, pH 7.8, 0.05 M NH₄Cl, 5 mM MgCl₂, 0.01 M NaCN, 1 mM dithiothreitol (buffer I); 8.5 A_{260} units of 50S particles, 0.20 μCi of ¹⁴C-methyl S-adenosyl-methionine (specific activity 55 $\mu\text{Ci } \mu\text{mol}^{-1}$) and with or without 15 μl of ribosome wash containing 50 μg protein. After 60 min at 37° C, reactions were stopped with 1.0 ml of a buffer containing 0.01 M Tris-HCl, pH 7.8, 0.05 M KCl, and 0.01 M MgCl₂ (buffer II); and the ribosomal particles were precipitated with 0.75 ml of cold ethanol⁹, and dissolved in 40 μl of 4 M urea and 2 M LiCl. After 16 h at 0° C, the ribosomal RNA was removed by centrifugation. The supernatant ribosomal proteins (about 100 μg proteins) were then electrophoresed¹³. After staining of the proteins by amido black, the gels were traced at 650 nm (—) using a linear transporter. The gels were then sliced and autoradiographed¹⁴ and the film was traced at 410 nm (---). a, The ribosome wash is absent and, b, the ribosome wash is present.

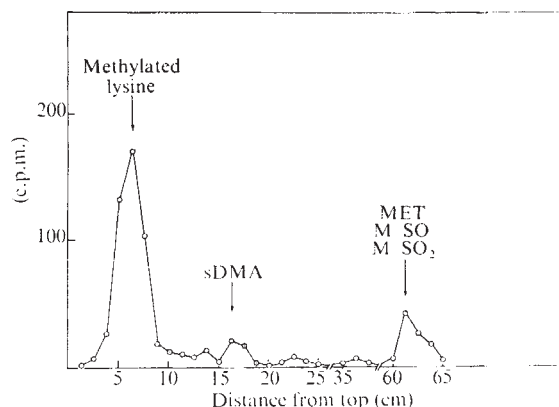


Fig. 2 Analysis of the methylated amino acids derived from protein L11 after *in vitro* methylation of the precursor 50S particle. The reaction conditions were the same as in Fig. 1 except 40 A_{260} units of the 50S particle and 1 μCi of ¹⁴C-methyl S-adenosyl-methionine in 0.7 ml of buffer I were reacted for 60 min at 37° C. The reaction was stopped with 2.0 ml of buffer II and the ribosomal proteins prepared according to the acetic acid procedure of Hardy *et al.*¹⁵. The ribosomal proteins were lyophilised to dryness and dissolved in 0.15 ml of the sample gel solution used for the first dimension run (pH 8.6) of the 2-dimensional electrophoresis procedure of Kaltschmidt and Wittmann¹². After electrophoresis, protein L11 was cut out from the gel and extracted for 16 h at 30° C with 0.01 M sodium phosphate buffer containing 0.1% sodium dodecyl sulphate (SDS). This was followed by dialysis against three changes of water to remove the excess dye and SDS. The dialysate was filtered to remove the gel and then lyophilised to dryness. The protein was then hydrolysed with 2 ml of redistilled 6 N HCl in a sealed tube at 110° C for 24 h. The hydrolysate was evaporated to dryness under reduced pressure and redissolved in 2 ml of water, followed by evaporation again to dryness. The final pellet was dissolved in 40 μl of water. 20 μl of the above sample were applied to a Whatman No. 3MM (20 × 110 cm) paper together with 10 μg of each of the following standard methylated amino acids: ϵ -monomethyllysine (MML), ϵ -dimethyllysine (DML), ϵ -trimethyllysine (TML), monomethylarginine (MMA), N^G,N^G-dimethylarginine (uDMA), N^G,N^G-dimethylarginine (sDMA), 1-methylhistidine (1-MH) and 3-methylhistidine (3-MH). The electrophoresis was carried out in a buffer containing pyridine-acetic acid-water (25:1:225), pH 6.5, for 3.5 at 4,440 V. After electrophoresis, the paper was sprayed with 0.4% ninhydrin in acetone and the methylated amino acids located by heating for 5 min at 70° C. The oxidised forms of methionine—methionine sulphoxide (M-SO) and methionine sulphone (M-SO₂) migrated with methionine (Met). The paper was then cut into 1.25-cm pieces and counted in a scintillation counter after the addition of 5 ml toluene-based scintillation fluid (4 g Omnifluor per litre toluene).

major methylated protein(s) comigrated with a major protein peak at 3.5 cm from the top of the gel. After the addition of the enzymes, methylation of two protein bands at about 2.0 cm from the top was observed (Fig. 1b). The latter methylation was dependent upon previous starvation of the culture with methionine as the 50S particle derived from the culture which was not starved with methionine was only methylated to a very small extent in the same conditions.

It was of interest therefore to identify the proteins from the 50S subunit that were methylated *in vitro*. The two-dimensional polyacrylamide gel electrophoresis procedure of Kaltschmidt and Wittmann¹² was used. More than 80% of the incorporated methyl groups was located in protein L11. Proteins L1, L3 and L5 were also labelled to a small extent. Independently, Alix and Hayes⁷ have also observed the *in vitro* methylation of protein L11 in cells grown in the presence of methionine—a methionine analogue.

The nature of the methylated amino acids in protein L11 was also determined. Figure 2 shows protein L11 contains methylated lysine(s) and a very small amount of a compound tentatively identified as N^G,N^G-dimethylarginine (sDMA). The nature of the methylated lysine was further

characterised to be ϵ -trimethyllysine. The presence of ϵ -trimethyllysine was also verified by the use of the amino acid analyser (C.N.C., and F.N.C., unpublished). It should be mentioned that we have also observed the presence of these two methylated basic amino acids in protein L11 obtained from cells grown with ^{14}C -methyl methionine⁶. Since the other proteins (L1, L3 and L5) were methylated to a much lesser extent, we have not been able to identify their methylated amino acids.

An attempt was also made to calculate the stoichiometry of ϵ -trimethyllysine in protein following *in vitro* methylation. In the *in vitro* experiment about 55% of the 8,000 c.p.m. incorporated into the 50S particle (8.5 A_{260} units) was found in the protein fraction. As 20% of the isolated *rel*⁻ 50S particles was in the precursor form which was predominately methylated (Fig. 1), the amount of incorporation of the methyl groups to protein L11 (which accounts for 80% of the total incorporation into proteins) can be calculated from the specific activity of ^{14}C -cethyl methionine (55 mCi mmol⁻¹) and 50% counting efficiency. It was found that there is about 0.30 molecules of ϵ -trimethyllysine per protein L11. This is lower than the value which we have observed for the *in vivo* methylation of L11 (0.8 molecules/L11) in exponential growth⁶. The discrepancy between these two results could arise because only a fraction of the precursor particle(s) was methylated or because there was a pool of methylated protein L11 present before the starvation which combined preferentially with the precursor 23S rRNA. This would lead to the lower incorporation of methyl groups to protein L11 *in vitro*.

We have recently shown that several ribosomal proteins form the 50S subunit (L1, L3, L5, L7, L9, L11, L12, L18 and L33) were methylated *in vitro*⁸. The failure to observe methylation of some of these proteins (such as L7, L9, L12, L18 and L33) *in vitro* in these experiments could either be due to these proteins being methylated already because they are essential for functioning, or due to the lack of methylases which specifically recognise these amino acids in the above proteins. These possibilities are now under investigation. Thus, the 50S particle obtained from *rel*⁻ strain after starvation for methionine should provide a good substrate for the assaying and eventual characterisation of protein methylases.

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RNA–RNA hybridisation using ^{125}I -labelled RNA from tobacco necrosis virus and its satellite

SINCE the description by Commerford¹ of a simple method of iodinating nucleic acids, RNA labelled *in vitro* with ^{125}I has been used in structural studies², and in DNA–RNA hybridisation studies *in vitro*³ and *in situ*⁴. This technique has considerable potential, especially in studying RNAs which are difficult to label *in vivo*, but most of the information available concerns the labelling of small RNA molecules and there is no information about the stability of large molecules under conditions necessary to obtain high specific activity. Furthermore, a recent report⁵ suggests that ^{125}I -labelled RNA with high specific activity is degraded on storage and its ability to form DNA–RNA hybrids is lost.

Here we show that large plant virus RNA species can be

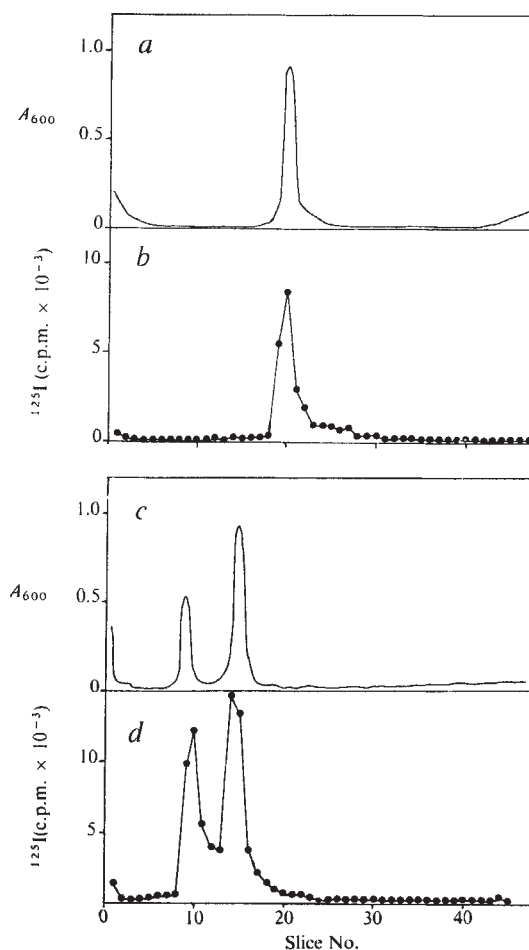


Fig. 1 Polyacrylamide gel electrophoresis of RNAs before and after ^{125}I -labelling *a*, SV RNA; *b*, SV RNA labelled for 20 min; *c*, TRV RNA; *d*, TRV RNA labelled for 2 min. Labelled and unlabelled RNAs were electrophoresed in parallel for 2 h in 8 cm, 2.4% acrylamide, 0.5% agarose gels containing 0.2% SDS and at 7.5 V cm⁻¹ as described by Peacock and Dingman⁹. Gels were stained for 1 h in 0.1% toluidene blue in 40% methoxyethanol and destained in 30% methoxyethanol. The isotope in the gel slices was determined by scintillation counting.

labelled with ^{125}I to high specific activities without significant degradation, that such molecules hybridise fully with complementary strand RNA and that these properties are unaffected by storage. We have used this technique to search for nucleotide sequence homology between the RNAs of satellite virus (SV) and its helper, tobacco necrosis virus (TNV)⁸.

Conditions for *in vitro* iodination of RNA were adapted from those described by Commerford as follows. The reaction mixture in a total volume of 25 μl contained 5 μg RNA, 5×10^{-4} μmol KI, 1.2×10^{-4} μmol TCI_3 , 1 mCi Na ^{125}I (carrier free) and 2.5 μmol sodium acetate, pH 4.8. The iodination was at 60° C for 20 min or for shorter periods for very large RNA species which were more susceptible to degradation. The reaction was stopped by making the mixture 0.01 M with respect to sodium sulphite, and 0.5 ml 0.05 M Tris, 0.1 M NaCl, 5 mM EDTA was immediately added. Yeast RNA (100 μg) was added as carrier and the RNA precipitated by addition of 2 vol ethanol. The precipitate was dissolved in 0.25 ml 0.1 M ammonium acetate, pH 8.9, and heated at 60° C for 10 min to remove unstable derivatives¹. After addition of 0.25 ml 0.05 M Tris, 0.1 M NaCl, 5 mM EDTA, pH 7.2, the RNA was precipitated with alcohol. The RNA was further purified by adsorption in 0.01 M potassium phosphate, pH 6.8, to a 0.5 ml column of hydroxyapatite, followed by elution with 0.25 M potassium phosphate. The overall recovery of RNA was approximately 50%.

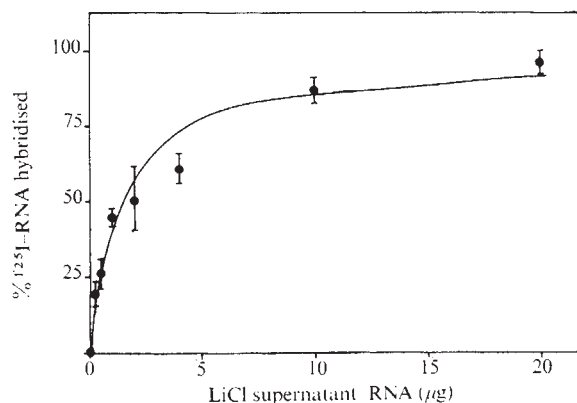


Fig. 2 Hybridisation of ^{125}I -labelled SV RNA with denatured double-stranded SV RNA. RNA was extracted from leaves of tobacco plants 3 d after infection with TNV strain A and SV1 (ref. 10) as described previously¹¹. High molecular weight single-stranded RNA was removed by LiCl precipitation¹² and the supernatant used as a source of double-stranded virus RNA¹¹. This RNA was mixed with 8,000 c.p.m. ^{125}I -labelled SV RNA to a total volume of 0.2 ml in $2 \times \text{SSC}$, and denatured by heating at 115° C for 15 min. Annealing was for 20 h at 70° C. TCA-precipitable ^{125}I was measured after ribonuclease digestion¹¹. Each point is the mean of three determinations. The solid line drawn is theoretical, and describes the relationship between the concentration of denatured double-stranded RNA and the fraction of single-stranded RNA hybridised under conditions where the reaction is complete¹³.

Using this method iodination for 20 min yielded RNA with a specific activity in the range 3×10^7 to 8×10^7 d.p.m. μg^{-1} . Iodination for 2 min gave RNA with a specific activity between 5×10^6 and 3×10^7 d.p.m. μg^{-1} . Figure 1 shows the electrophoretic behaviour of these RNAs on polyacrylamide gels before and after iodination. SV RNA (molecular weight 0.4×10^6)⁷ is undergraded after iodination for 20 min. The two RNAs of tobacco rattle virus (molecular weights 2.5×10^6 , 0.7×10^6) (ref. 8) were essentially intact after iodination for 2 min, but the larger of the two RNAs was considerably degraded during a 20 min labelling period. The behaviour of the iodinated RNA on acrylamide gel electrophoresis was unaltered after storage for 3 months as an ethanol precipitate at -20° C.

The ability of these labelled RNAs to hybridise with comple-

mentary strand RNA was examined by annealing them with denatured double-stranded virus RNA extracted from virus-infected plants. Figure 2 shows the results of annealing ^{125}I -RNA of SV strain 1 with increasing concentrations of denatured double-stranded SV1 RNA. At high concentrations of double-stranded RNA nearly all the ^{125}I is ribonuclease resistant. The data fit well to the line drawn, which is theoretical and describes the relationship between the concentration of denatured double-stranded RNA and the fraction of single-stranded RNA hybridised under conditions where the reaction is complete¹³. The results shown in Fig. 2 were obtained with ^{125}I -RNA which had been stored for 3 months.

These techniques have been used to look for common nucleotide sequences in TNV RNA and SV RNA. The total dependence of satellite virus on TNV for replication⁶ is thought to be due to a requirement by SV RNA for the RNA-dependent RNA polymerase specified by TNV and it has been suggested that TNV RNA and SV RNA may contain common nucleotide sequences which act as recognition sites for such a shared polymerase¹⁴. The two RNAs are known to contain an identical trinucleotide at their 5' termini¹⁴⁻¹⁶, and Klein and Reichmann¹⁷ reported that at least 10% of the SV RNA genome was homologous with that of TNV.

In an experiment in which ^{125}I -labelled SV RNA was hybridised to denatured double-stranded SV RNA, in the absence of any competitor RNA, approximately 3,000 c.p.m. of ^{125}I -labelled RNA was hybridised. When unlabelled SV RNA (10^{-13} mol) was included as a competitor, hybridisation was reduced by about 90%, whereas the same molar concentration

Table 1 Attempts to form hybrids between SV RNA and TNV RNA complementary strand

Source of double-stranded RNA	^{125}I -labelled RNA (c.p.m.)	^{125}I -hybridised (c.p.m.)
Uninfected plants	TNV (8,650)	151 $\pm$ 23 (3)
TNV infected plants	TNV (8,650)	2070 $\pm$ 120 (3)
Uninfected plants	SV (2,330)	43 $\pm$ 2.7 (6)
TNV infected plants	SV (2,330)	38 $\pm$ 1.6 (6)

Denatured LiCl supernatant RNA (100 μg) from either uninfected or TNV infected plants was annealed with ^{125}I -labelled SV or TNV RNAs, the iodinated RNAs being used at the same molar concentrations. Experimental methods were as described in Fig. 1. The mean values for the quantities of ^{125}I hybridised are given with their standard errors, and the figures in brackets in this column refer to the number of replicates in each case. No detectable ^{125}I -labelled SV RNA was annealed by TNV complementary strand RNA ($< 0.5\%$ $P > 0.99$).

of TNV RNA had no detectable effect, indicating a lack of sequence homology between these RNAs. A more sensitive approach is to attempt to anneal ^{125}I -labelled SV RNA with denatured double-stranded TNV RNA (Table 1). Less than 0.5% of the ^{125}I -labelled SV RNA hybridised to TNV complementary strand RNA in this experiment. Since this concentration of TNV complementary strand was sufficient to anneal with 23% of the ^{125}I -labelled TNV RNA it follows that less than 2% of the SV RNA sequences are homologous with TNV RNA sequences. Any sequence homology which may exist between these two RNAs is therefore limited to a total of less than 30 nucleotides, and this is difficult to reconcile with an earlier report of considerable sequence homology between these RNAs¹⁷.

We have shown here that iodinated RNA has considerable potential in RNA-RNA hybridisation studies. It should be particularly useful to plant virologists since pure virus RNAs are often readily available, but isotopically labelled RNA is particularly difficult to obtain by *in vivo* labelling methods.

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Double helix formed by the dinucleoside phosphate thymidyl-3',5'-deoxyadenosine

IN our conformational studies of nucleic acid components we have obtained X-ray diffraction photographs from specimens of the dinucleoside phosphate thymidyl-3',5'-deoxyadenosine (TpdA) which are similar to those obtained from certain synthetic DNA copolymers. The dinucleoside phosphate was obtained from International Enzymes Ltd as the triethylammonium salt. Some of it was dissolved in a small quantity of water between a glass slide and a coverslip resting on two thin glass fibres. Some of the material which dried out under the coverslip showed evidence of orientation when viewed between crossed polaroids. X-ray diffraction photographs were taken on a microcamera using a Hilger and Watts semi-microfocus X-ray generator and one of these is shown in Fig. 1.

The diffraction patterns are similar to those obtained from fibre specimens of nucleic acids and they are characteristic of helical diffraction patterns, with an integral number of residues per turn of the helix. The layer-line spacing at ~57% relative humidity is 26.3 Å. There are discrete reflections only on the equator and these can be indexed on a hexagonal lattice with $a=21.25$ Å. Both layer-line spacing and the equatorial pattern depend on water content and at ~92% relative humidity the layer-line spacing is 27.2 Å and $a=22.7$ Å. The pattern is characterised by a very strong intensity on the seventh layer line close to the meridian, by a strong first layer line and a weaker second layer line. These characteristics are similar to those observed in some of the fibre X-ray diffraction patterns of the synthetic double-helical copolymers poly d(A-T)-poly d(A-T) (ref. 1) and poly d(I-C)-poly d(I-C) (ref. 2).

The similarity in the diffraction patterns obtained with TpdA and the polymers can be explained if two TpdA molecules are related to each other by a twofold rotation axis so that they are linked to each other by Watson-Crick pairing of bases in a similar way to that observed in the single-crystal structure of the dinucleoside phosphates adenylyl-3',5'-uridine (ref. 3) and guanylyl-3',5'-cytidine (ref. 4). Pairs of TpdA molecules can then stack on top of each other to produce an extended helical structure. The structure would thus be similar to a double helix in which alternate phosphate groups would be missing. The fact that the backbone of such a helix is discontinuous suggests that

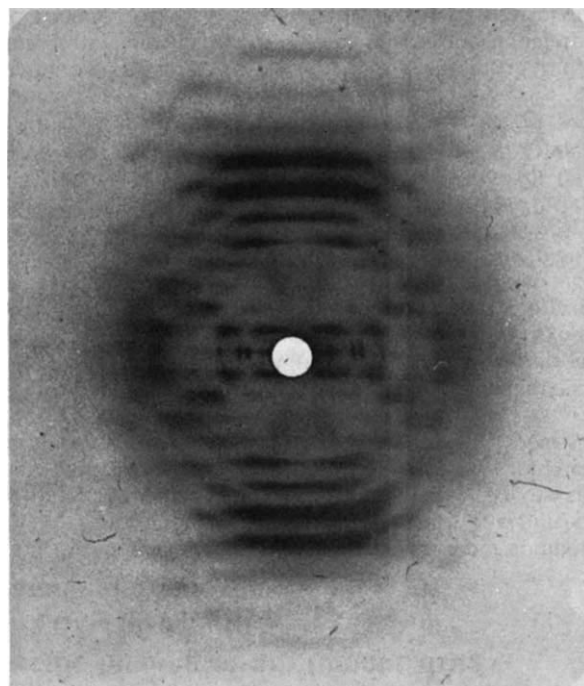


Fig. 1 X-ray diffraction photograph of TpdA at 57% relative humidity.

stacking forces are important in stabilising the structure and in determining its integrity.

The intensity distribution on the fifth, sixth, seventh and eighth layer lines is similar in some ways to the distribution in the corresponding region of the diffraction pattern of the A-form of DNA⁵, suggesting that the bases are tilted from perpendicular to the helix axis. The tilt is 20° in A-DNA, where the rise per nucleotide along the helix is 2.56 Å. In TpdA, an estimate of the number of molecules in the 26.3 Å repeat gives a value of 12 or 10,—an odd number of molecules would mean a doubling of the repeat period which is not observed. Twelve molecules would correspond to a stack of six pairs of molecules with a rise of 2.19 Å from one base to the next along the helix. Ten molecules correspond to a stack of five pairs of molecules and a rise of 2.63 Å, which seems a more reasonable value when compared with polynucleotide structures. Further analysis of the diffraction patterns should clarify this point and should enable a detailed structure determination to be made. It will be of interest to compare the TpdA structure with that recently proposed for poly d(A-T)-poly d(A-T) (ref. 6).

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Specific binding of antitumour drug *cis*-Pt(NH₃)₂Cl₂ to DNA rich in guanine and cytosine

ONE of the more effective *cis*-platinum antitumour compounds currently being tested in cancer chemotherapy is *cis*-Pt(NH₃)₂Cl₂ (ref. 1). Evidence suggests that DNA is the effective site of action of the platinum drug^{2,3}. This study was initiated to determine the effective binding site on DNA. Roberts and Pascoe have noted the similarity of action between *cis*-Pt(NH₃)₂Cl₂ and the classical antitumour bifunctional alkylating agents which crosslink DNA (ref. 2). Low concentrations of several *cis*-platinum compounds suppress DNA synthesis *in vitro* while affecting protein and RNA synthesis to a much lesser extent³. The studies by Horacek and Drobnik employing ultraviolet absorption spectroscopy indicate that the platinum moiety binds to the bases of DNA rather than to the phosphate backbone⁴. Spectroscopic studies suggest that *cis*-Pt(NH₃)₂Cl₂ preferentially binds to guanine-cytosine (G-C)-rich DNA^{5,6}. An increase in the buoyant density of T7 DNA and calf thymus DNA has been reported when one of several platinum compounds is present in solution^{5,7}. Here we report that the binding of *cis*-Pt(NH₃)₂Cl₂ to DNA results in large increases in buoyant density which are proportional to the G-C content of the DNA and to the molar ratio of platinum to DNA-P.

Rat liver DNA (42% G-C), radiolabelled with ³H-thymidine, and *M. lysodeikticus* DNA (72% G-C), both prepared by standard methods⁸, were mixed in 0.015 M sodium chloride. Concentrations of the original DNA solutions were determined using the molar extinction coefficients at 260 nm of 6650 for rat liver DNA and 6700 for *M. lysodeikticus* DNA (ref. 9). Solutions containing 10⁻³ M *cis*-Pt(NH₃)₂Cl₂ in 0.015 M sodium chloride were added and the mixture incubated at room temperature in the dark. Buffers were avoided because of their potential metal-binding properties¹⁰. Periodic measurements during the course of the reaction indicated a pH of about 6.5. The ultraviolet absorption parameter $A_{250\text{ nm}}/A_{270\text{ nm}}$, an indication of platinum binding to DNA (ref. 4), was used to monitor the reaction. This ratio decreases until equilibrium is established. In the conditions employed, this required about 1 d.

Aliquots containing 10-30 µg of platinised DNA were then mixed with caesium sulphate solution and banded in an isopycnic gradient. The refractive index and absorbance at 260 nm of sequential 0.3 ml fractions were determined and densities were derived using the formula of Vinograd¹¹. The radioactivity of each fraction was determined using Aquasol (New England Nuclear) and a Hewlett Packard liquid scintillation counter. Some gradient fractions were dialysed to remove the caesium salt and the ultraviolet absorption spectrum of the recovered DNA was determined. The ratio $A_{250\text{ nm}}/A_{270\text{ nm}}$ indicated that the molar ratio of DNA-bound platinum to DNA-P had remained unchanged during the isopycnic banding procedure.

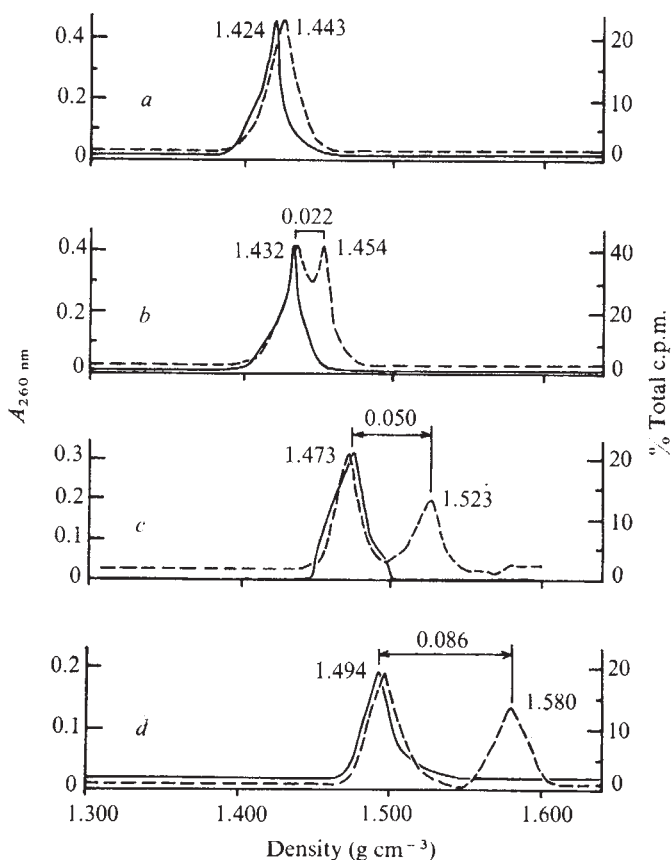


Fig. 1 Rat liver DNA, radiolabelled with ³H-thymidine, was mixed with *M. lysodeikticus* DNA, reacted with various amounts of *cis*-Pt(NH₃)₂Cl₂ and analysed in a caesium sulphate isopycnic density gradient. The gradients were fractionated and the refractive index, the absorbance at 260 nm (----) and the radioactivity (—) were determined as described in the text. The buoyant densities of unreacted rat liver DNA and *M. lysodeikticus* DNA were determined individually as 1.424 g cm⁻³ and 1.443 g cm⁻³ respectively. The concentrations of rat liver DNA, *M. lysodeikticus* DNA and *cis*-Pt(NH₃)₂Cl₂ used were a, 9.20 × 10⁻⁵ M (DNA-P), 1.17 × 10⁻⁴ M (DNA-P), 0; b, 8.40 × 10⁻⁵ M, 1.06 × 10⁻⁴ M, 9.90 × 10⁻⁶ M; c, 7.70 × 10⁻⁵ M, 9.72 × 10⁻⁵ M, 1.67 × 10⁻⁶ M; d, 6.93 × 10⁻⁵ M, 8.74 × 10⁻⁵ M, 2.57 × 10⁻⁶ M.

Figure 1 illustrates the increasing separation of rat liver DNA and *M. lysodeikticus* DNA at higher concentrations of *cis*-Pt(NH₃)₂Cl₂. *M. lysodeikticus* DNA increases in density more than ³H-thymidine-labelled rat liver DNA, which has a lower G-C content. To rule out the possibility that *M. lysodeikticus* DNA remained in the low density band, the experiment was repeated without rat liver DNA. We obtained a single homogeneous peak for *M. lysodeikticus* DNA and an increase in density similar to that obtained in the previous experiment. When the experiment is performed without *M. lysodeikticus* DNA a single homogeneous peak is also obtained for rat liver DNA.

The effects of *cis*-Pt(NH₃)₂Cl₂ on other DNAs were determined in a similar manner. The findings are presented in Fig. 2. The buoyant density increase of each DNA is proportional to its G-C content and also depends on the molar ratio of platinum to DNA-P. Similar results have been obtained using caesium chloride gradients.

We wished to determine how firmly the platinum compound was bound to the DNA. Exhaustive dialysis of platinised DNA against some of the usual chelating agents such as EDTA or penicillamine or in solutions containing high concentrations of such anions as sulphate or chloride failed to reverse the buoyant density and spectroscopic changes induced by treatment of DNA with the platinum compound. Preliminary

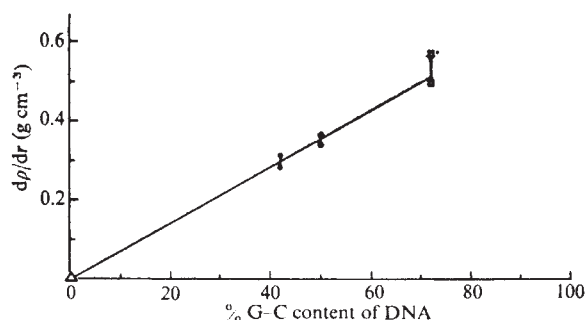


Fig. 2 The relationship between the increase in density of a DNA, treated with *cis*-Pt(NH₃)₂Cl₂, and the base composition of that DNA. The increase in buoyant density, ρ , of various DNAs as a function of the increase in the molar ratio of platinum to DNA-P, dr , is presented as dp/dr versus G-C content of the DNA. *Cis*-Pt(NH₃)₂Cl₂ was reacted with individual DNAs in 0.015 M sodium chloride to completion. The buoyant density was determined as described in the text and the value of dp/dr , the platinum dependent density change for the DNA, was obtained from a plot of buoyant density versus molar ratio of platinum to DNA-P. To determine whether or not dp/dr was altered by the presence of another DNA, a second procedure was used which yielded similar values. Equal amounts of a test DNA and a reference DNA, for which dp/dr was known, were reacted with *cis*-Pt(NH₃)₂Cl₂ in a 0.015 M sodium chloride solution. These DNAs were banded in caesium sulphate as described in the text. Then, $(dp/dr)_{\text{test DNA}} = (dp/dr)_{\text{reference DNA}} \times \frac{\Delta\rho_{\text{test DNA}}}{\Delta\rho_{\text{reference DNA}}}$. For example, from Fig. 1, the increase in buoyant density for *M. lysodeikticus* DNA is 1.8 times the increase in density for rat liver DNA from the same solution. Therefore: $(dp/dr)_{\text{M. lysodeikticus DNA}} = 0.30 \text{ g cm}^{-3} \times 1.8 = 0.54 \text{ g cm}^{-3}$. The value of 0.30 g cm^{-3} for rat liver DNA, in this case, was determined by the increments in density that occurred when this DNA alone was reacted with various concentrations of the platinum compound. Values of dp/dr were obtained for (poly (dA) · (poly (dT))) and poly (dA-T)³ (Δ) calf thymus DNA (42% G-C) and rat liver DNA (42% G-C) (●) *E. coli* DNA (50% G-C) (○), and *M. lysodeikticus* DNA (72% G-C, (□).

indications, however, are that most, but not all, of the DNA-bound platinum can be removed by dialysis for several weeks with 10^{-3} M solutions of potassium cyanide and 0.015 M sodium chloride. It is not presently known whether or not removal of the DNA-bound platinum results in nicking or other damage to the DNA.

Understanding of the action of *cis*-Pt(NH₃)₂Cl₂ may result in the development of more useful analogues of these anti-tumour agents. In addition, isolation of guanine rich regions of fragmented DNA might be facilitated using the larger increase in density which occurs with platinisation.

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RNase inhibition of reverse transcriptase activity in human milk

THE aetiological role of RNA tumour viruses in mammary cancer of experimental animals coupled with observations of morphologically similar particles in human milk^{1,2} has motivated considerable interest in the biological role of these virions in man. The specificity and sensitivity of the simultaneous detection test³ for reverse transcriptase (RT) which requires that the viral enzyme polymerise radioactive DNA using endogenous 70S and/or 35S viral DNA as template, suggested its usefulness for the detection of RNA tumour viruses in human milk⁴. In two recent studies^{2,5} comparing the detection of such virions in human milk by electron microscopy (negative staining) with the detection of RT activity, no correlation was observed. As a part of our effort at relating the presence of such virions in human milk to a familial history of breast cancer, we employed reconstruction experiments to determine the reliability of the simultaneous detection test for the routine testing of human milk samples for the presence of RNA tumour viruses. Analysis of the products of the reaction was carried out by the use of gel electrophoresis instead of by velocity gradients⁶. This method offers the advantage that, in addition to the 70S and/or 35S viral peak, it also detects a 4S peak which represents the DNA products of the reaction which are free from the large molecular weight viral RNA.

Using this modification of the simultaneous detection test, we have repeatedly and reproducibly detected the presence of RNA tumour viruses from a variety of sources including some samples of human milk. Experiments describing the high degree of proportionality and reproducibility readily attained with non-milk sources will be described elsewhere.

In our reconstruction experiments, 3×10^4 focus forming units (FFU) of murine leukaemia virus (MuLV) (Rauscher) supplied by University Laboratories and assayed by them in FFU of helper virus) was added to each of 25 human milk samples obtained from healthy donors. Under these conditions, any endogenous RT activity would be expected to contribute less than 10% of the total counts and thus not be a source of confusion. Figure 1 shows the electrophoretic profiles of the products of the simultaneous detection test. Profile *a* gives the products of the MuLV control diluted in 0.85% NaCl. Four of the 25 samples gave profiles identical to this profile. Profile *b* was seen in 17 of the 25 samples. There is no 35S peak and little or no 4S peak. Profiles *c* and *d* with reduced 35S and 4S peaks were seen in 4 out of 25 samples. Similar results were obtained when avian myeloblastosis virus (AMV) was substituted for MuLV in this series of reactions. It is clear, therefore, that the majority of the milk samples tested inhibited the detection of the reverse transcriptase reaction of at least two known RNA tumour viruses.

We had previously observed profiles similar to these in Fig. 1*b*, *c* and *d*, when, before beginning the enzymatic reaction, we had deliberately added RNase to the viral pellet to demonstrate the RNA dependency of the re-

action. Therefore, we made a direct examination for the presence of RNase in the viral pellet prepared from the milk samples described above. Table 1 shows the relationship between RNase activity in the viral pellet from milk and the inhibition of RT activity of the same pellet. The positive correlation between the presence of RNase in the viral pellet and the inhibition of the RT activity supports the suggestion that RNase in human milk is a significant inhibitor of the simultaneous detection test for RT. It was not possible to examine these particular milk samples further since the limited amount of milk available was used in these assays.

A similar interference with the RT activity of MMTV in milk from RIII mice by an inhibitor present in human milk was reported earlier⁴. These workers also reported that on some occasions trypsin pretreatment could be shown to increase the RT activity detectable in human milk samples. We tested trypsin pretreatment of human

milk containing added MuLV and found that it rarely prevented inhibition of RT. Similarly we found that it failed to lower the measured level of RNase present in such milk samples (J. J. McC., L. J. L., and M. A. R., unpublished).

Since the simultaneous detection test was inadequate for screening milk samples, we investigated whether another RT assay might offer a valid approach. The use of exogenous synthetic templates, such as poly (A)-oligo (dT) and poly (dA)-oligo (dT) or poly (C)-oligo (dG) (ref. 7), to assay RT activity provided an extremely sensitive assay, but this reaction was also inhibited when reconstruction experiments with MuLV or AMV added to milk were carried out and the test run on the viral pellet.

Table 1 RNase and reverse transcriptase in human milk seeded with MuLV

Milk no.	RNase	RT activity (% of control)
1	+	0
2	+	20
3	+	43
4	+	0
5	+	0
6	+	13
7	+	24
8	+	87
9	+	0
10	+	0
11	+	0
12	—	100
13	—	100
14	—	100
15	+	0
16	+	0
17	+	0
18	+	0
19	+	0
20	+	0
21	+	0
22	+	0
23	+	0
24	+	0
25	+	0

Reverse transcriptase activity (% control) was determined as in Fig. 1. The control was 3×10^4 FFU of MuLV seeded into 15 ml of 0.85% NaCl. To determine the presence of RNase activity, 0.005 ml of the viral pellet immediately after resuspension in 0.050 ml of 10 mM Tris-HCl (pH 8.3) was added to tritiated mouse ribosomal RNA and incubated at 37° C for 1 h. Glycerol was added to a concentration of 10% and the sample subjected to electrophoresis on a 1.8% acrylamide 0.5% agarose gel for 60 min at 2 mA per gel. Gel sections were cut, hydrolysed in 30% peroxide at 60° C and counted in Aquasol in a Packard liquid scintillation spectrometer. A sample was designated RNase negative if a sharp 28S RNA peak containing 75% or more of counts of the control was found. The 28S peak was absent from all samples designated RNase positive.

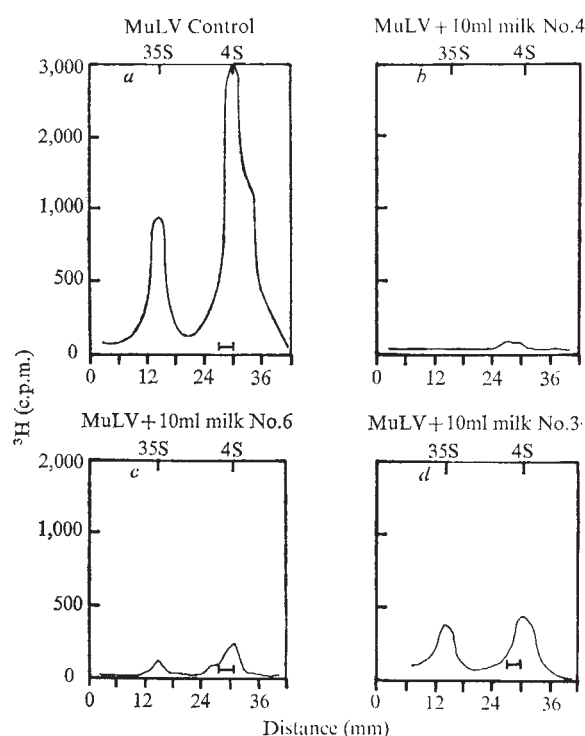


Fig. 1 Twenty five human milk samples were 'seeded' with 3×10^4 FFU of MuLV assayed for RT activity according to the procedure of Schlom *et al.*⁴, (with the minor modifications described below) and the products analysed by polyacrylamide gel electrophoresis⁶. The peak between fraction 12 and 14 represents the 35S viral RNA which is acting as template in the reaction. The peak between fractions 24 and 36 represent the DNA products of the reaction which are no longer bound to the 35S RNA. Four of the 25 samples gave profiles identical to that in *a* (MuLV assayed independently of milk), 17 of the 25 gave profiles like *b*, and 4 gave profiles like *c* and *d*. 15 ml of milk containing added MuLV was diluted with 7 ml of 10 mM Tris-HCl, 150 mM NaCl, 2 mM MgCl₂ (pH 7.4) (TNM) and centrifuged at 4,000g for 10 min. The clear 'milk-plasma' zone between the lipid and the pellet was removed and overlaid on a 8 ml glycerol pad and centrifuged at 4° C for 1 h at 98,000g in a Spinco SW 27 rotor to form the 'viral pellet.' Centrifugation of the milk-TNM mixture at 4,000g for 10 min and of this supernatant at 16,300g for 10 min to clear the large molecular weight material before the centrifugation at 98,000g was not effective in enhancing the reverse-transcriptase activity of the viral pellet or of lowering the RNase activity of this pellet. The use of EDTA instead of TNM did not aid in the precipitation of casein in human milk and inhibited the RT reaction of MuLV or AMV as has been noted¹². The RT assay was carried out with an incubation at 37° C for 1 h (identical curves showing about 25% as much incorporation were obtained with 15 min incubations).

The other type of reverse transcriptase assay in common use is the endogenous template method⁸. This is a less refined version of the simultaneous test in which all of the radioactive products of the reaction are precipitated together. Such a method would also be unsatisfactory for the assay of MuLV or AMV seeded human milk samples since, as shown in Fig. 1, in 84% of the samples tested the total amount of radioactive product is significantly lower than the control value.

If RNase present in human milk is, indeed, inhibiting the reverse transcriptase activity of virus added to such a sample, it should be possible to demonstrate this by determining the amount of RNase present and then showing that adding this amount of RNase to a reverse transcriptase reaction in buffer causes the same amount of inhibition as adding the milk sample itself. To determine

the amount of RNase present in the reverse transcriptase reactions of a number of milk samples, we centrifuged them, as described for Fig. 1, using the procedures we employed for preparing 'viral pellets'. We resuspended such pellets in 0.01 M Tris-HCl pH 8.3 in 1/100 the original volume and determined the amount of RNase present in aliquots of this solution using ribosomal RNA as described in Fig. 2. We then determined how much inhibition of a virus RT reaction in buffer, using the template poly (C)-oligo (dG), was caused by a particular volume of this resuspended milk pellet. To equate this inhibition with the concentration of RNase present, equivalent concentrations of pancreatic RNase were added to a parallel series of viral RT reactions and the percentage inhibition determined. Since mouse mammary tumour virus (MMTV) may be a better model for a human mammary virus than leukaemia virus, these assays were done with MMTV purified from RIII mouse milk⁸ as well as with MuLV. The results are shown in Fig. 2.

Milk samples 33 and 39 show the same degree of inhibition of the RT reaction with MMTV or MuLV as was obtained when the equivalent amount of pancreatic RNase was added to the MMTV RT reaction. Low levels

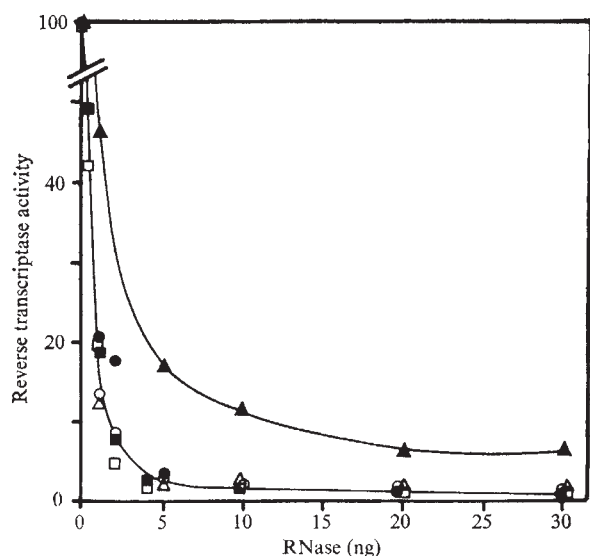


Fig. 2 The inhibition of a synthetic template reverse transcriptase reaction by the addition of pancreatic RNase or milk 'viral pellet' which contains the same amount of RNase. The human milk was prepared as described in the legend of Fig. 1, and the 'viral pellet' resuspended in 10 mM Tris-HCl (pH 8.3) in 1/100 the original volume. RNase was assayed quantitatively by measuring the TCA-precipitable counts after incubating tritiated mouse ribosomal RNA with the aliquots of the resuspended 'viral pellet'. Incubation was for 10 min at 0°C. 10% TCA was added to stop the reaction and 50 µg of yeast RNA was added as carrier. Samples were precipitated on cellulose nitrate filters, dried, and counted in a toluene-PPO-POPOP cocktail in a Packard liquid scintillation spectrometer. The units of RNase per test sample were determined by comparison with the TCA precipitable counts remaining after exposure of the RNA to a known amount of commercial bovine pancreatic RNase A (80 Kunitz units mg⁻¹) (Worthington Biochemical Corporation, Freehold, New Jersey). Aliquots of ▲, pancreatic RNase (dissolved in 10 mM Tris-HCl, pH 8.3); ●, the resuspended 'viral pellet' of human milk sample 2. 33 (2 ng of RNase ml⁻¹ of milk used) ■, human milk no. 39 (10 ng of RNase ml⁻¹ of milk used), each containing equal amounts of RNase activity, were added to RT assays containing 3×10^3 FFU of MuLV and 5 µg ml⁻¹ of the synthetic template (poly rC):oligo (dG) and the reaction carried out as described. An identical series of reactions was also carried out with MMTV in the RT assay (△, ○, □). The fact that a similar inhibition of the reaction is seen when identical levels of RNase are added demonstrates that the RNase present in the milk 'viral pellet' is sufficient to explain the inhibition of such reactions seen when MuLV, AMV or MMTV are added to human milk in reconstruction experiments.

of pancreatic RNase added to MuLV show a similar RT inhibition, but the RT appears to be more resistant to higher RNase concentrations. The viral pellet of sample 33 contains 2 ng RNase ml⁻¹ of milk used to prepare the pellet and sample 39 contains 10 ng RNase ml⁻¹ of milk used. From these data it is clear that the inhibition of RT activity by human milk samples can be accounted for by the RNase activity of the viral pellets.

It is not immediately clear how RNase, a small molecular weight protein, can reach the viral pellet since the centrifugation conditions (98,000g for 1 h over a 8 ml pad of 20 or 30% glycerol) are clearly not sufficient to pellet RNase. We have noted, however, that the milk samples centrifuged in these conditions form a large pellet. Since casein, a common milk protein, can form micelles of sufficient mass to pellet in these conditions, we examined the viral pellet for casein by the Ouchterlony double diffusion technique against rabbit anti-human casein. Human casein was readily detected in these pellets suggesting that RNase could indeed reach the viral pellet as part of a casein micelle.

We have found that the amount of RNase in the resuspended 'viral pellet' is proportional to the volume of human milk used and that the variation of RNase present in a group of milk samples tested was between 2 and 20 ng ml⁻¹ (1 mg of RNase is equal to 80 Kunitz units (ref. 9)). In the synthetic template RT assay shown in Fig. 2, 3.0×10^3 FFU of MuLV virus, which we have shown by laser light scattering contains about 0.5×10^7 viral particles¹⁰, is 95% inhibited by 2.5 ng of RNase. Assuming that the RT activity of the virus found in human milk using this template is as sensitive to RNase as MuLV or MMTV, a human milk sample would have to contain more than 0.5×10^7 endogenous viral particles ml⁻¹ if it contained 2 ng of RNase ml⁻¹ and more than 1×10^8 particles if it contained 20 ng of RNase ml⁻¹ to exhibit positive RT activity with a synthetic template test. We have verified these figures by the addition of suitable amounts of MuLV to human milk and found that when virus is in excess, positive RT's are obtained as predicted.

The milk of various strains, especially RIII, is well known to contain large amounts of MMTV and such samples have routinely given positive RT assays. We have found that the RNase activity of the 'viral pellets' of RIII mouse milk falls in the same range as that from human milk samples, but that the viral content of such samples as determined by laser light scattering is 25–500 times higher than the amount of virus added to human milk in our reconstruction experiments. It is clear that the large difference in the number of viral particles can account for the high frequency of positive RT assays with RIII mouse milk as opposed to human milk.

The data presented here may well account for the lack of correlation between RT activity in human milk, and the detection of virus particles by electron microscopy, or the epidemiological risk to breast cancer^{2,5,11}. Attempts to assess the RNase sensitivity of endogenous RT activity in human milk and to develop an inhibitor or RNase compatible with the detection of RT activity are in progress.

We thank Dr Jeffrey Schlom for setting up the simultaneous test in our laboratory, Ms Ruth Rich for help, Dr Sam Albert under whose direction the milk was acquired, and Dr Herbert Rose for carrying out immunological determinations.

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Capacity of complement C3 phenotypes to bind on to mononuclear cells in man

SINCE the discovery of the genetic polymorphism of C3, the third component of human serum complement¹⁻³, no demonstration of functional differences between the common phenotypes F and S has been published. Total or specific C3 haemolytic activity has been found to be the same for C3 variants^{1,4} as was also the case with immune adherence titres in sera with different C3 phenotypes¹.

Evidence has accumulated about the complement receptors in the membrane of leukocytes in various animal species including man^{5,6}. The complement component involved in the binding to the leukocytes seems to be C3 (ref. 5). It seemed worthwhile investigating the possibility of genetically determined differences in C3 molecules showing in this potentially significant immunological function of C3. Here evidence is put forward that there are functional differences in man between the common phenotypes F and S in the capacity to bind with the complement receptor on mononuclear cells *in vitro*.

Mononuclear cells from normal human individuals were obtained by Ficoll-Isopaque centrifugation of defibrinated peripheral blood and washed twice by centrifugation at 200g with Hanks's basal salt solution (HBSS) and resuspended in HBSS. C3 phenotypes of healthy laboratory staff and blood donors were determined by starch gel electrophoresis as described previously⁷. Complement was coupled on to erythrocytes by two methods. First, the method of Jondal *et al.*⁸ was used. Human blood group O red cells were coated with antibody by incubating with inactivated rabbit anti human O red cell serum diluted 1:1000. For the binding of complement, human sera at a dilution of 1:10 were incubated with the erythrocyte-antibody complex (HEAC-technique). Secondly, low ionic strength was used in order to coat red cells with complement⁹. One millilitre of 5% human erythrocyte suspension in saline was spun down and the supernatant discarded. One drop of fresh human serum was added to the packed cells followed by 1 ml 10% sucrose (w/v). The mixture was incubated at 37°C for 15 min. Then the cells were washed three times with saline and finally suspended in 2 ml of HBSS (HEC-technique).

Table 1 The effect of C3 phenotype on the number of HEC rosette-forming mononuclear cells and the concentration of serum C3

	C3 phenotype	
	S	F
No. of sera studied	9	10
HEC-rosettes (mean %)	3.7	6.4
Total no. of HEC rosettes counted	294	521 ($\chi^2 = 63.64$)
Total no. of mononuclear cells counted	3,966	4,061 ($P < 0.001$)
C3 concentration in serum (mg per 100 ml, mean $\pm$ s.d.)	73.3 $\pm$ 12.2	68.1 $\pm$ 25.1

For the preparation of rosettes, 0.5×10^6 mononuclear cells and 0.1 ml of 2.5% complement coated red cell suspension were mixed in a final volume of HBSS of 0.5 ml and centrifuged at 200g for 5 min. A drop of 0.02% acridin orange was added in the tube and the mixture was resuspended for 2 min on a slowly rocking mixer. The counting of the rosettes was performed in a Hawksley Cristallite haemocytometer under an ultraviolet light microscope where the nucleated cells could easily be distinguished by their yellow stain. All the determinations were made in duplicate. To minimise the possible effect of day to day variation, an approximately equal number of F and S sera were tested in each experiment. The concentration of serum C3 was determined by radial immunodiffusion¹⁰ (M-Partigen C3c Behringwerke, Marburg, Germany).

In the first phase of the study, sera were investigated which had been frozen for 3 yr. With the HEAC-technique, 10 C3F and 11 C3S sera were tested and the mean percentages of rosette-forming cells were 9.6 and 7.5%, respectively.

Because of the difference it seemed worthwhile to investigate the matter further and fresh serum samples from individuals typed previously for C3 phenotypes were requested. The results from this material are shown in Table 1 and Fig. 1. The HEC-technique was utilised because, with the antiserum used for sensitising red cells in the HEAC technique, the background rosettes (that is, rosettes obtained with antibody coated erythrocytes) were nearly as numerous as the complement rosettes. By coupling the complement on to the erythrocytes with low ionic strength the interference caused possibly by the antibodies and the respective receptors on monocytes was avoided. The amount of antibody coupled on to the erythrocytes with this technique was nil or negligible; this was indicated by the fact that no rosettes were obtained

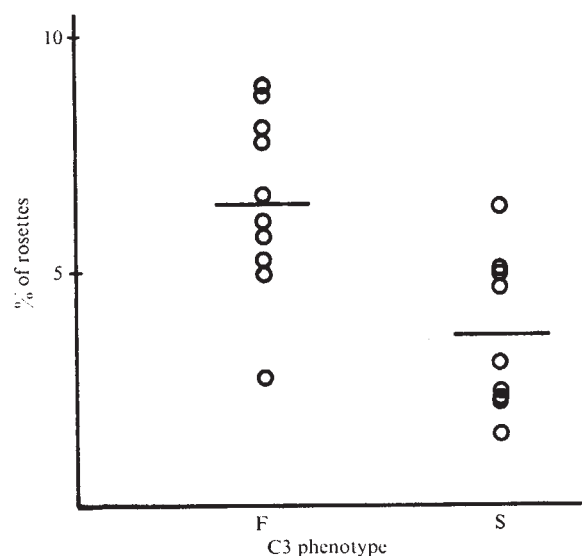


Fig. 1 Influence of serum C3 phenotype on the number of HEC rosette-forming mononuclear cells. Each point represents the mean of duplicate determinations. The mononuclear cells used in this series of experiments originated from a single donor of C3S phenotype. The difference is statistically significant ($P < 0.01$) by Finney's first analogue of Fieller's theorem¹⁷.

with red cells treated with fresh inactivated sera. Furthermore, no immunoglobulins could be shown on the red cells with antiglobulin technique. The result observed here could have arisen from differences in *in vitro* conversion between C3 F and S. This is unlikely, however, as no such difference has been found previously^{11,12}. This possibility was further studied with fresh serum samples from two C3F and two C3S individuals. The results of the experiment, completed within 5 h from the moment of venepuncture, were comparable to those in Table 1. The possibility of C3F and S being coupled on to the erythrocytes in different amounts is unlikely, too, because the red cells treated with doubling dilutions of C3F and S sera were agglutinated to similar titres with anti-complement serum. It seems most probable that, compared to C3S, C3F has an enhanced capacity to bind with the respective receptor on mononuclear cells.

Preliminary attempts were made to classify the cells which exhibited the receptor for complement with the present method. The number of rosettes did not correlate with the number of polymorphonuclear cells in experiments with buffy coat leukocytes. In two experiments where mononuclear cells were allowed to adhere to a plastic surface and were separated subsequently into adherent and nonadherent cells the HEC rosette forming cells were enriched into the former cells. The mean figures were 8.4% in the initial cells suspension, 30.6% in the subpopulation of adherent cells and 1.2% in that of nonadherent cells. Moreover, the majority of the cells able to form HEC-rosettes seem to possess the capacity of phagocytosing particles (unpublished results). These findings imply that the cell in question may be monocyte.

In European populations the frequencies of homozygotes for C3^S and C3^F are roughly 60–70% and 3–5% respectively⁷. My observation that C3F and C3S differ in the capacity to bind on to mononuclear cells is interesting from several points of view. First, C3 seems to be one of the few genetically polymorphic human serum proteins where functional differences between the variants have been demonstrated. Because a lot is known about the biological function of C3 in body defence mechanisms this protein might deserve a closer look by geneticists, especially the supporters of the selectionist view¹³.

Second, the nature of the receptor site on the membrane of the leukocytes is virtually unknown. Studies with C3F and S molecules might shed more light on properties of the C3 receptor on mononuclear cells. Also C3 phenotype may be another variable in studies where human complement-coated erythrocytes are used for detecting subpopulations of mononuclear cells.

Thirdly, of particular interest in this connection are the findings of Brönneham¹⁴ and Farhud *et al.*¹⁵. They observed independently a significant surplus of C3^F gene among patients with rheumatoid arthritis. Furthermore, an association between C3^S gene and high titres of anti-A or anti-B in newly delivered mothers¹⁶ is another observation suggesting that C3 phenotype may have an influence on various immunological processes *in vivo*. This present *in vitro* finding might offer a clue to explaining the mechanism of these phenomena.

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Factors influencing microculture of leukaemia cells

THE culture of normal haemopoietic cells was accomplished¹ by obtaining colonies of granulocytic and monocytic cells from mouse bone marrow grown in a semisolid agar system. The method was modified for human bone marrow and subsequent studies showed that leukaemia cells from the peripheral blood and marrow of patients with acute myelogenous leukaemia (AML) produced small colonies or clusters^{1,2}. Using this technique, serial sampling and morphological or biochemical studies of the growing cells was difficult. A liquid culture technique was then developed to study the growth of granulocytic or monocytic cells from normal bone marrow³. This *in vitro* diffusion chamber technique had the advantage of easy sampling but involved the use of large culture vessels. The method has subsequently been used to cultivate AML cells⁴.

We describe here a new short term microculture technique for growing AML cells in suspension which overcomes the cumbersome nature of the above methods and facilitates the study of multiple factors influencing the growth of these cells. The method is simple, reproducible and does not require the addition of a colony stimulating factor.

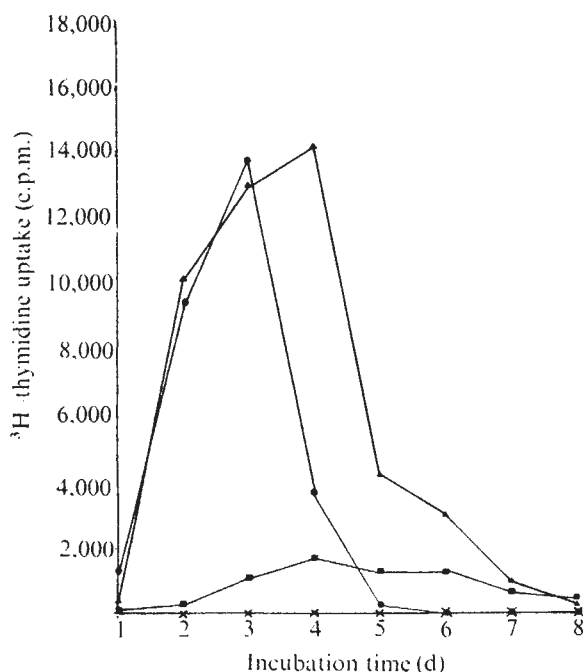


Fig. 1 The optimum cell number and time of collection for the microculture of AML cells. x, 1×10^3 ; ■, 1×10^6 ; ▲, 1×10^7 and ●, 1×10^8 cells ml^{-1} .

Both fresh and liquid nitrogen-stored leukaemia cells have been grown successfully in this system. Red cells were removed from fresh heparinised blood by the addition of a 2% solution of methyl cellulose to accelerate their sedimentation and the white cell layer washed once. Leukaemia cells were collected from the peripheral blood of patients with AML using an NCI IBM cell separator and were stored with 10% dimethylsulphoxide in liquid nitrogen. For culture, these cells were thawed rapidly, diluted slowly with RPMI 1640 medium (Biocult Limited) and then washed. After washing, the cells were resuspended at a concentration of $1 \times 10^6 \text{ ml}^{-1}$ in medium 199 (Wellcome) containing 0.3 ml of L-asparagine, 6.6 mg ml^{-1} , and 10% foetal calf serum (Flow Laboratories) or 10% AB serum. Aliquots of this suspension (0.2 ml) were added to microculture plates (Falcon 3040). Following 3 d incubation in a humid 5% CO_2 atmosphere the growth of these cells was measured by the addition of 0.1 mCi ^3H -thymidine (specific activity 5 Ci mmol^{-1}) to each well for 16 h. The trichloroacetic acid precipitable material was collected on filter paper and counted in a liquid scintillation counter.

Some samples of leukaemia cells stored in liquid nitrogen showed low viability. A method of slow dilution of 2 ml of original cell suspension to 20 ml over 10 min greatly improved the viability of the cells as measured by trypan blue exclusion, the incorporation of tritiated thymidine in liquid microculture and colony formation in semisolid agar. The results are shown in Table 1.

A wide range of media were tested using both fresh and stored cells from several patients, and it was found that Medium 199 (Wellcome) supplemented with 10% serum and L-asparagine produced the best growth. The

special stains (Sudan black and periodic acid-Schiff) were consistent with this diagnosis. Cultures have also been obtained using peripheral blood cells from patients with chronic granulocytic leukaemia. In these instances maturation to myelocytes and segmented forms was characteristic. Leukaemia cells from the blood of ten patients with acute lymphoblastic leukaemia failed to grow using this system.

This raises the possibility of using the method for diagnostic purposes in patients with acute leukaemia. A relatively easy, objective test, distinguishing acute myelogenous and lymphoblastic leukaemia would be of great value. Our results suggest that this method may be useful in this context. A study of patients with undifferentiated leukaemia is now being made.

Using the microculture technique, we have shown that sera from AML patients immunised during remission with allogenic irradiated leukaemia cells can cause growth inhibition and cell death in cultures of allogenic AML cells. This property is complement dependent and is probably due to anti-HLA antibody which is known to be present in these sera using conventional assays. One patient in remission with AML has been found to have complement-dependent growth inhibitory activity against autologous stored leukaemia cells. In this experiment the autologous remission serum was not inhibitory in the absence of complement, but the addition of complement to the autologous serum resulted in a reduction of 80% in uptake of tritiated thymidine. Complement alone did not significantly affect the uptake of thymidine. Further studies to determine the frequency of this finding are under way.

We feel that this new technique is of value in a number of contexts; first, as an objective, diagnostic test to distinguish acute myelogenous and lymphoblastic leukaemia; second, in studies of serum factors influencing the growth of AML cells and, third, in studies of *in vitro* sensitivity to chemotherapeutic agents.

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Table 1 Relationship between rate of dilution and viability of AML cells stored in liquid nitrogen

Rate of dilution	Initial viability	Plating efficiency (agar)	Uptake of ^3H Tdr (liquid culture)
Fast	64%	0.4%	6,052 6,118
			6,165 6,656 (6,007)
			5,401 5,652
			9,913 11,728
Slow	80%	3.0%	10,936 12,731 (11,562)
			12,570 11,491

results depicted in Fig. 1 show that after an initial decline the cell concentration increased to a peak on day 4 or day 5 of culture and that DNA synthesis, as measured by the uptake of tritiated thymidine, reached a peak on day 3 or day 4 of culture. This experiment also showed that the cell concentration for optimal growth in this system was $1 \times 10^6 \text{ ml}^{-1}$. Autoradiographs of cells obtained from these cultures showed that labelled cells had the morphological and cytochemical characteristics of AML cells. This technique has therefore been shown to measure a true proliferation of AML cells in short-term culture, with an increase in cell number as well as thymidine incorporation.

More than 25 samples of acute myeloblastic and myelomonoblastic leukaemia cells have been grown from fresh blood with complete success. The success rate for growing liquid nitrogen-stored cells was approximately 80% but this is now increasing as our storage techniques have improved. The AML cells which multiplied in liquid culture did not mature in the normal manner. After 4 d the cultures consisted almost entirely of myeloblasts and promyelocytes or primitive monocytoïd cells. Some small lymphocytes were also present. The cultured cells from patients with acute myeloblastic leukaemia were nevertheless usually easily identified as being of the granulocytic series. The typical granulation was often prominent and

Membrane antigens of murine leukaemia cells

THE membranes of lymphoid cells from tumours induced by oncornaviruses are antigenically altered. The new surface antigen(s) are readily recognised by isologous (histocompatible) hosts which mount both cellular and humoral immune responses^{1,2}. It is essential to know whether or not antigens induced by the various strains of murine leukaemia viruses (MuLV) are cross-reacting immunologically.

The first thorough serological study, made in a completely isologous system, was done by Old *et al*³. They compared the cell membrane antigens induced by Friend virus complex (MuLV-F), Rauscher virus complex (MuLV-R), Moloney leukaemia virus (MuLV-M) and Gross virus (MuLV-G) using cytotoxic antisera from inbred mice. Similarity was noted between antigens induced by Friend, Rauscher and Moloney viruses and a single cross-reacting antigenic system designated FMR was described. This

peritoneal injections of the respective virus (0.05–0.2 ml of 1% w/v, cell-free, spleen homogenate). Leukaemia cells were obtained from spleens (for MuLV-F and MuLV-R leukaemias) or from lymph nodes (for MuLV-M leukaemia). In addition, MuLV-M leukaemia cells were obtained as an established YAC cell line derived from A/HeJ mice⁸ and grown in our laboratory in suspension with McCoy's 5a medium and 20% foetal bovine serum.

Antisera to MuLV-F-induced cell membrane antigen,

Table 1 Cell membrane immunofluorescence test with various isologous antisera*

Experiment	Antiserum†	Dilution	Leukaemia target cells (% stained)			MuLV-M§
			MuLV-F	MuLV-R	MuLV-M‡	
A	anti-FVMA/1	1:4	> 85	> 85	50	
		1:8	> 85	> 85	< 15	
		1:32	> 85	60	< 15	
	anti-FV/L	1:4	> 85	> 85	80	
		1:8	> 85	> 85	< 15	
		1:32	50	40	< 15	
	anti-MVMA/1	1:4	< 15	< 15	> 85	> 85
		1:32	< 15	< 15	> 85	
	Normal serum	undiluted	< 15	< 15	< 15	< 15
		undiluted	> 90	> 90	> 90	
B	anti-FVMA/2	1:2	> 90		70	
		1:4	> 90		40	
		1:8	> 90		< 10§	
		1:32 to 1:128	> 90		< 10	
		1:256	50			
		1:512	< 10			
	anti-MVMA/2	undiluted	35		> 90	
		1:2	< 10		> 90	
		1:4 to 1:256	< 10		> 90	
		1:512			80	
		1:1024			50	
		1:2048			< 10	

* For each dilution, 0.035 ml of serum was added to a pellet of washed leukaemic lymphocytes (10⁶) from the above preparation. After incubation at 37° C for 30 min, the cells were again washed twice with buffered salt solution (Dulbecco's BSS, GIBCO) and resuspended in fluorescein-conjugated goat anti-mouse globulin (Hyland) diluted 1:15 in BSS. The tubes were then incubated again at 37° C for 30 min, washed twice with BSS and resuspended in 50% glycerol-BSS for storage. Cells were then examined within one week using a Zeiss microscope with fluorescent optics and a BG 12 filter. For controls tested with normal mouse serum, the number of cells positive with anti-globulin in the absence of specific anti-MA did not exceed 10–15%.

† Two different pools of antisera to MuLV-F cells membrane antigen (anti-FVMA/1 and 2) and of antisera to MuLV-M cells membrane antigen (anti-MVMA/1 and 2) were tested. Anti-FV/L is an isologous (BALB/c) antiserum cytotoxic for Friend leukaemia cells. Normal serum was pooled from BALB/c mice.

‡ YAC cell line.

§ Lymph node cells from leukaemic mice.

|| Percentage of cells with traces of fluorescence was as low or lower than the background (10% or 15%) with normal serum.

antigen was not found on the cells of Gross leukaemia and, conversely, antiserum prepared against Gross leukaemia had no activity for FMR. This classification was readily accepted by other investigators in the field⁴.

We were, therefore, surprised to find that a battery of various isologous antisera against MuLV-F leukaemic cells prepared in our laboratory in the course of two years gave relatively little cross-reaction with MuLV-M leukaemia cells and vice versa. Subsequently we learned that another investigator had a similar experience (F. Lilly, unpublished). Here we report our observations suggesting similarity between MuLV-F and MuLV-R cellular antigens and their distinction from MuLV-M antigen(s) and we present a careful look at the previous data concerning the 'FMR antigen' concept.

MuLV-F originally purchased from the American Type Collection (Bethesda, Maryland) was obtained from Dr W. Ceglowski of Penn State University; its previous passage history on BALB/c mice was reported⁵. In our laboratory, this virus was also passaged in BALB/c (ref 6). It is equally infectious for BALB/c and Swiss/ICR mice. MuLV-R also passaged on BALB/c, was obtained originally from pool No. 46 AP-RLV. MuLV-M, described in detail elsewhere⁷ was supplied by Dr M. R. Proffitt (Massachusetts General Hospital, Boston).

Leukaemias were induced in young adult BALB/c mice (Charles River Laboratories, Wilmington, Mass.) by intra-

FVMA, were prepared by immunisation of BALB/c mice with formaldehyde virus vaccine⁹ and with several intra-peritoneal injections of isologous viable leukaemic spleen cells. Another anti-FVMA serum, anti-FV/L (Tables 1 and 2) was prepared in BALB/c mice by injection of live N-tropic MuLV-F followed by boosts with increasing doses of live NB-tropic virus. Antiserum to MuLV-M induced cell membrane antigen (MVMA) was prepared in A/HeJ mice by immunisation with the YAC cell line (derived from A/HeJ mice). The first injection of irradiated cells (10⁶, 6,000 rad, cobalt 60, 320 rad s⁻¹) was followed by several additional intraperitoneal injections of 10⁷ live YAC cells.

The specific activity of antisera was tested by indirect membrane fluorescence¹⁰ using fresh leukaemia target cells. Anti-FVMA sera reacted strongly with the membrane of MuLV-F cells and (to a lesser degree) with MuLV-R cells; 85 to 100% cells expressed the antigen (Table 1). Anti-FV/L serum (prepared in the former manner showed a similar specificity for MuLV-F and MuLV-R cells. In contrast, these sera reacted very weakly, and only at high concentrations, with MuLV-M cells. The cross reactivity disappeared at dilutions of 1:8 or higher. The stronger of the antisera, anti-FVMA/2, had a titre of 256 with MuLV-F cells and only a titre of 4 with MuLV-M cells (Table 1, experiment B). The isologous anti-MuLV-M serum seemed to be even more specific: pool 2 had a titre of 1,024

against MuLV-M cells and a titre of 2 with MuLV-F and MuLV-R membrane antigens.

Cross-absorption studies supported the seemingly close relationship between MuLV-F and MuLV-R membrane antigens and their distinction from MuLV-M antigen (Table 2). The reactivity of anti-FVMA sera with homologous Friend cells was completely abolished by absorption with either MuLV-F or MuLV-R cells but was not diminished (at the given concentration of the antiserum) by absorption with MuLV-M cells. Conversely, the specific activity of anti-MuLV-M serum was absorbed with MuLV-M cells but not with MuLV-F or MuLV-R cells.

These results argue against the concept of FMR as the

Gross leukaemia cells in the membrane fluorescence test but not in cytotoxic assay¹⁰.

It seems that the number of antigen types found on the surface of leukaemia cells may be much more complex than previously thought. In addition to the virus induced antigens^{14,15}, these might include virus envelope antigens^{16,17}, virus core antigens¹⁸, foetal antigens^{18,19} and other specificities.^{20,21} Our observations, as well as these reports, suggest that the 'FMR' term is probably misleading and it should be interpreted with caution.

We thank Dr G. Klein, of the Karolinska Institute, Stockholm, and Dr F. Lilly, of the Albert Einstein College of Medicine, New York, for encouragement. MuLV-R was

Table 2 Cell membrane immunofluorescence with cross-absorbed antisera to murine leukaemias*

Serum	Absorbed (cells)	Leukaemia Target Cells (% stained)		
		MuLV-F	MuLV-R	MuLV-M
a-FVMA/1	MuLV-F	<10	<10	<10
	MuLV-R	25	<10	<10
	MuLV-M	>90	>90	<10
	Normal (control)†	>90	>90	<10
a-FV/Lilly	MuLV-F	<10	<10	<10
	MuLV-R	<10	<10	<10
	MuLV-M	85	>90	<10
	Normal (control)†	85	>90	<10
a-MVMA ±	MuLV-F	<10	<10	>90
	MuLV-R	20	<10	>90
	MuLV-M	<10	<10	<10
	Normal (control)†	<10	<10	>90

*Absorption was done in three steps using equal volume of packed cells (washed thoroughly in BSS and preincubated with normal BALB/c serum for 40 min at room temperature to minimise a nonspecific loss of antibody) and undiluted antisera; 20 min at 37° C bath, 45 min at room temperature and overnight at 4° C. Cells from the same source were used for both absorption and immunofluorescence assay (MuLV-M cells were YAC).

† Sera were absorbed with pooled, normal (non-infected) BALB/c spleen cells.

major cell surface antigen common for the three leukaemias. A careful evaluation of previously published work, in which the relationship between the murine leukaemia cellular antigens was studied using either serological techniques or transplantation resistance, seems to support our criticism. Glynn *et al.*¹¹ and Bianco *et al.*¹² reported that BALB/c mice were protected against transplantation of Moloney lymphomas by pre-treatment with syngeneic MuLV-M cells but not with MuLV-R leukaemia cells, suggesting a lack of cross-reactivity between the transplantation antigens of the two leukaemias. Using similar techniques, Glynn *et al.* later reported positive finding of cross-reactivity between the F, R and M leukaemias induced in C57 B1/6 mice¹³. We found, however, that the data in their experiments showed a close relationship between F and R, but some separate specificity for M. Thus, mice pretreated with MuLV-F or MuLV-R leukaemia cells were completely resistant to Friend lymphoma, whereas immunisation with MuLV-M cells conferred only a partial protection which did not seem to be much different from the protection conferred by Gross leukaemia cells¹³ (Table 5).

Although it can not be assumed that the antigens responsible for transplantation immunity are necessarily the same as those involved in serological tests, the latter appears to correlate with the transplantation studies discussed above. As employed by Glynn *et al.*¹³, membrane fluorescence showed a degree of cross-reactivity between F, M and R antigens, but there was an indication for existence of FR and M sub-groups at higher serum dilutions.

The cytotoxic assays of Old *et al.*³ showed clear distinction between G and FMR antigens but results were not as clear for equally strong cross-reactivity between F, R and M because titres of antisera (particularly following *in vivo* absorption) were as low as 2-4. In this respect it is interesting that Klein and Klein found high titred antisera to Moloney leukaemias which reacted rather well with

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Autologous human B and T lymphoblastoid cell lines

HUMAN lymphoblastoid cell lines (LCLs) have been derived from patients with acute and chronic leukaemias as well as from normal individuals¹⁻³. Most of these LCLs express characteristics of bone marrow-derived (B) lymphocytes^{4,5} and contain the Epstein-Barr virus (EBV) genome⁶ and the EBV-determined nuclear antigen (EBNA)⁷. Cell lines derived from the blood of patients with myelogenous leukaemia also contain B lymphocyte markers^{4,8}, which may indicate that they are not of leukaemic origin. In patients with lymphatic leukaemia, it has been less clear whether a derived LCL represents the neoplastic cell population. Recently, LCLs derived from patients with acute lymphoblastic leukaemia were found to possess characteristics of thymus-derived (T) lymphocytes^{3,5,9}.

We have studied two LCLs, both derived from the same blood specimen of an 11-yr-old boy with lymphoblastic lymphoma which progressed to an acute leukaemic phase. Their isolation has been previously described^{10,11}. We report here that these two lines displayed very different biological properties. One line, CCRF-SB (here designated SB) expressed B-lymphocyte characteristics, had a human diploid karyotype, contained the EBV genome and EBNA, and activated normal allogeneic lymphocytes in the mixed lymphocyte reaction (MLR) to proliferate and develop cytotoxic capability. In contrast, the other line, H-SB2, expressed T-lymphocyte characteristics, had an abnormal human karyotype, lacked detectable EBV genome and EBNA, and did not activate allogeneic lymphocytes. These and other observations to be discussed suggest that the T-LCL is derived from the malignant lymphoblast population.

SB and H-SB2 were examined for HL-A antigens and for the presence of five independent markers of B and T lymphocytes, as described previously^{6,12}. B-lymphocyte markers included surface immunoglobulin (Sig), complement C3 receptor (CR), and the human B lymphocyte-specific surface antigen (HBLA). T-lymphocyte markers included the sheep red blood cell (SRBC) receptor and the human T-lymphocyte-specific surface antigen (HTLA). EBV genome was detected by DNA-DNA reassociation kinetics analysis¹³ and EBNA by anti-complement immunofluorescence⁷. The capacity of the LCLs to stimulate

normal peripheral lymphocytes in a one-way MLR was tested as previously reported¹⁴. In addition, after culture for 6 d with either SB or H-SB2, these peripheral blood lymphocytes were tested for their ability to kill SB and H-SB2 in a 4 or 5 h ⁵¹Cr-release assay previously described¹⁵.

Both SB and H-SB2 grow in suspension culture and have the morphological appearance of lymphoblasts. Originally, SB cells were diploid, whereas, H-SB2 cells displayed F-group trisomy in approximately 50% of the cells¹⁶. In addition, both lines were found to share common HL-A antigens (A₁, A₂, A₁₂, W₁₇). Table 1 summarises our findings.

Tests for T and B lymphocyte markers revealed that SB expressed B-cell characteristics while H-SB2 expressed T-cell characteristics. Virtually all SB cells had Sig and HBLA, 15-75% formed rosettes with C3 coated SRBC, and all lacked T-cell markers. In contrast, all H-SB2 cells had HTLA, 20-60% formed rosettes with unsensitized SRBC and all lacked B-cell markers.

Tests for the presence of EBV (Table 2) demonstrated that all SB cells contained EBNA and that by DNA-DNA re-naturation kinetics analysis the cell line contained eight genome equivalents of EBV DNA per cell. H-SB2 lacked EBNA and was negative for EBV genome at the limit of the test sensitivity which was 0.2 genome equivalents per cell.

The MLR tests (Table 3) revealed that SB activated the lymphocytes of three donors with stimulation indices ranging from 24.0 to 39.5, whereas, H-SB2 failed to activate lymphocytes from the same donors. H-SB2 did not inhibit the response to SB when both cells were used together as stimulators, demonstrating that the absent MLR response to H-SB2 was due to a lack of stimulation capacity rather than active inhibition by H-SB2. Furthermore, SB but not H-SB2 was found to generate significant cytotoxic effector cells. Lymphocytes activated by SB killed both SB and H-SB2 equally well, however, presumably on the basis of cross-reactive antigens.

The heterotransplantability of SB and H-SB2 has been studied by Adams, *et al.*¹⁹. They discovered that H-SB2 could be successfully transplanted in normal neonatal Syrian hamsters, whereas SB could not be transplanted unless the recipient hamsters were first immunosuppressed with anti-lymphocyte serum. In addition, H-SB2 tumours progressed to a leukaemic phase in 5% of the animals bearing tumours, whereas SB tumours never progressed to a leukaemic phase. Perhaps the capacity of H-SB2 to proliferate in the untreated hamster is related to the failure of these cells to stimulate lymphocytes in the MLR.

The proliferative and cytotoxic effector phases of the MLR can serve as models of immune recognition and cell-mediated immunity (CMI) *in vivo*¹⁷. It has been suggested that the *in vitro* MLR represents the first stage of the immunological surveillance mechanism which ultimately removes abnormal lymphoid cells which arise *in vivo*¹⁸. Therefore, the failure of H-SB2 to elicit CMI responses *in vitro* may have biological significance with respect to the *in vivo* surveillance of certain T cell malignancies.

The results reported here extend previous observations^{19,20} suggesting that SB and H-SB2 may be derived from different lymphoid cell populations. SB had B-lymphocyte surface markers and a normal karyotype, whereas, H-SB2 had T-lymphocyte surface markers and an abnormal karyotype. In addition,

Table 1 Characteristics of SB and H-SB2 cell lines

Cells	% of cells with B-lymphocyte markers*			% of cells with T-lymphocyte markers*		Presence of EBV		Stimulation of lymphocytes <i>in vitro</i>	
	Sig	CR	HBLA	SRBC	HTLA	Genome	EBNA	Proliferative response	Cytotoxic response
SB	90-100	15-75	100	0	0	+	+	+	+
H-SB2	0	0	0	20-60	100	-	-	-	-

Sig, Surface immunoglobulin; CR, complement receptor; HBLA, Human B lymphocyte antigen; SRBC, sheep red blood cell (receptor); HTLA, human T lymphocyte antigen.

* Day-to-day variation occurs with some tests and is indicated by a range of values.

Table 2 Presence of EBV genome and EBNA

Cells	No. of EBV genome equivalents per cell*	% of cells showing EBNA†
SB	8	100
H-SB2	<0.2	0
Negative control (Calf thymus DNA)	<0.2	
Positive control (Raji cell line)	60	100

* DNA-DNA reassociation kinetics analyses were carried out as previously described¹³ with 0.01 µg of ³H-EBV DNA (2.7 × 10⁶ c.p.m. µg⁻¹) and 2 mg of sonicated cell DNA extracted from the cultured lymphoblasts. The portion of DNA reassociated was determined by hydroxyapatite column chromatography and the number of EBV genome equivalents calculated as described^{13,26}. EBV was purified from the extracellular fluid of the HR1K cell line.

† The EBNA test was a modification of the anti-complement immunofluorescence technique described by Reedman and Klein⁷. Acetone-methanol fixed cells were treated with a 1:1 mixture of human serum containing antibody against EBNA and fresh human complement obtained from an individual negative for EBV antibody (final dilution 1:10 for each). After incubation at 37°C for 45 min., the smears were washed and treated with a 1:10 diluted fluorescein isothiocyanate-conjugated anti-human β₂C/β₁A globulin at 37°C for 45 min. EBNA positive cells did not react with control serum lacking antibody to EBNA.

SB carried the EBV genome and EBNA, could not grow in normal neonatal hamsters, and could activate normal lymphocytes *in vitro* to proliferate and develop cytotoxic capability. Similar properties have been found for other LCLs derived from normal people^{6,7,19,21}. In contrast, H-SB2 lacked detectable EBV genome, grew as tumours in normal newborn hamsters, and failed to activate lymphocytes to proliferate or to become sensitised killer cells. Other T-LCLs, MOLT-4 (ref. 3), CCRF-CEM (ref. 1), and RPMI-8402 (ref. 22), have displayed these same properties when examined^{14,22-27} and have been derived only from patients with acute lymphoblastic leukaemia.

Table 3 DNA synthesis and cytotoxic effect of lymphocytes activated by SB and H-SB2 in the mixed lymphocyte reaction

Activation mixture*	³ H-thymidine incorporation CPM	Stimulation index†	% ⁵¹ Cr release‡ ±s.e. from SB	% ⁵¹ Cr release‡ ±s.e. from H-SB2
A+SB _m	82,501	24.0	68.3±3.3	59.1±0.6
A+H-SB2 _m	2,430	0.7	1.0±0.1	-17.7±0.9
B+SB _m	122,075	39.5	52.8±13.7	75.0±2.2
B+H-SB2 _m	3,390	1.1	-11.8±0.4	-1.6±0.3
C+SB _m	108,932	31.0	91.3±3.3	84.0±6.4
C+H-SB2 _m	2,222	0.4	0.5±0.3	13.1±0.6

DNA synthesis was measured on day 6 in a one-way MLR, as previously described¹⁴. Each reaction mixture contained 2.0 × 10⁵ responding lymphocytes and 0.5 × 10⁵ stimulating LCLs treated with mitomycin C. The cytotoxic effect of the stimulated lymphocytes was measured on day 6 after setting up activation mixtures, as previously described¹⁶. Cytotoxicity was measured by incubating 1.0 × 10⁶ attacking lymphocytes with 1.0 × 10⁴ ⁵¹Cr-labelled target cells in RPMI 1640 medium containing 10% heat-inactivated foetal calf serum for 4 or 5 h at 37°C. The cells were then centrifuged and the supernatant aliquots removed for counting of ⁵¹Cr release. Controls consisted of target cells incubated with cultured but non-sensitised lymphocytes from the appropriate donor. Maximum ⁵¹Cr release was obtained by freeze-thawing the labelled target cells in distilled water three times.

* A, B, and C, peripheral blood lymphocytes from three donors; SB_m and H-SB2_m, mitomycin C-treated LCLs of these lines.

† Values are ratios of the mean c.p.m. of ³H-thymidine uptake in the stimulated lymphocytes and the mean c.p.m. in the unstimulated lymphocytes (cultured alone).

‡ Values are corrected for ⁵¹Cr release from control cultures.

SB was probably derived, as are virtually all B-LCLs of normal people, from EBV containing normal B lymphocytes that spontaneously transform during *in vitro* culture. H-SB2, on the other hand, was probably derived from the malignant T lymphoblasts of the leukaemic donor. By analogy, the other known T-LCLs of leukaemic patients are probably also of neoplastic origin.

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reviews

ONE of the most intriguing questions that can be asked about the development of science is why it took place in Europe between 1250 and 1650 and not in any of the great civilisations of antiquity. Ancient Babylon, Egypt and China, to take but three examples, enjoyed long periods of relative stability and developed the technological arts to a very high degree, but of science as we know it there were hardly more than a few scattered beginnings. The Greeks came nearest with the mathematics of Euclid, the physics of Archimedes, the astronomy of Aristarchus and the accurate biological observations of Aristotle, but in spite of all this the enterprise never really got off the ground and soon it was all forgotten, to be rediscovered later and developed in the Middle Ages.

Dr Jaki's book is a detailed examination of this problem. He surveys all these civilisations as well as those of the Hindus, the Incas, the Aztecs and the Mayas to see if it is possible to isolate the reason for the many stillbirths of science before its final birth in Europe. He finds that, in one way or another, all the earlier civilisations had a cyclic conception of the universe. In spite of its evident absurdity this idea, with every event repeating itself exactly at regular intervals an infinite number of times, has exerted a perennial fascination on man. It has always brought with it deep pessimism and despair, a sense of the futility of

Science needed Christianity

P. E. Hodgson

Science and Creation. By S. L. Jaki. Pp. vii+367. (Scottish Academic Press; Edinburgh and London, July 1974.) £4.50.

all effort, sapping all confidence in steady progress. Why try to discover anything if it is destined all to be forgotten and then discovered again, eternally? In addition, matter was often viewed as the battleground between capricious deities, and in such an atmosphere science could not develop at all.

Although we seldom recognise it, scientific research requires certain basic beliefs about the order and rationality of matter, and its accessibility to the human mind, that are seldom found in ancient civilisations. They came to us in their full force through the Judaeo-Christian belief in an omnipotent God, creator and sustainer of all things. In such a world view it becomes sensible to try and understand the world, and this is the fundamental reason why science developed as it did in the Middle Ages in Christian Europe, culminating in the brilliant achievements of the seventeenth century. This time there

was no miscarriage, and science has flourished ever since as one of the most robust movements of thought the world has ever seen.

The old ideas died hard, and the history of the first 15 centuries of our era is the history of a continuous struggle between the old and the new. The early Christians were not directly concerned with paving the way for science yet this was one of the results of their labours. St Basil was a relentless critic of astrology, and St Augustine established the intellectual framework of an intelligible creation that enabled science to emerge centuries later. The early mediaevalists, notably Adelard of Bath, insisted that a rational explanation be sought for natural phenomena and More's *Utopia* articulated the ideas that later flowered in the science of Galileo, Kepler, Copernicus and Newton.

Dr Jaki develops this thesis with impressive erudition, chronicling in great detail the false starts, fantastic speculations, and occasional brilliant insights of philosophers in a wide range of cultures. It is very salutary to retrace these forgotten and stumbling steps, for thereby we can appreciate more clearly the magnitude of the achievements of those who at last succeeded in hammering out the basic concepts of science as we know it, and showed how to develop and refine them by a systematic programme of reflection and experimentation. □

BIOMECHANICS seems to have become a popular subject, and the appearance of another book on the subject might be greeted with some suspicion. Were this another textbook, such suspicion might be justified, but it is nothing of the sort. It is, as the author says, a highly personal, individualistic discussion of a few of the biomechanical problems on which he has worked. There are just five chapters; one is introductory, the others consider in turn egg-eating snakes, legless locomotion (by snakes, amphisbaenians, and caecilians), burrowing by amphisbaenians, and anuran respiratory/vocal systems. Fish, birds, and mammals get scant attention, and invertebrates none at all, yet these omissions are scarcely noticed, so interesting is the book.

Each chapter commences by posing the obvious questions that a functional anatomist would ask, proceeds to find

Three in one

D. W. Yalden

Biomechanics. By Carl Gans. Pp. x+261. (Lippincott: Philadelphia; May, 1974.) Cloth: \$12.50; paper: \$5.95.

answers to them, and continues with the further questions that these answers throw up; it includes discussion of the limitations both of the answers and of the methods used to obtain them. Obviously, the treatment of the topics is thorough, yet the book is by no means couched in technical language or otherwise difficult to understand. The wealth of illustrations (over 150) which include explanatory graphs and complex anatomical drawings, and superb photographs, clarifies and considerably enhances the book.

This is indeed a most worthwhile

book on at least three levels. Though not stated explicitly, it is in fact a herpetology book, and useful because of, for example, its treatment of some of the more obscure reptile groups. Second, it is indeed a book of biomechanics, and has useful points to make on the application of physical principles to some quite unexpected facets of animal life. Third, it is a methodology book, and I feel that for this reason in particular it deserves to be read by every zoology undergraduate who has the slightest interest in whole animal zoology. Perhaps too, it ought to be read by anyone retaining the conceited view that all can be explained at the molecular level. Here is the mind of a modern zoologist at work, combining time-honoured methods of comparative anatomy and new-fangled instrumentation in an attempt to understand how animals live and work.

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Bony philosophy

Concepts and Approaches in Animal Morphology. By P. Dullemeijer. Pp. xii+264. (Van Gorcum: Assen, Netherlands, 1974.) n.p.

BIOLOGISTS who think about the philosophical basis of their work usually stop doing so rather quickly and scurry back to the laboratory. Their unease is caused mainly by the historical and purposive aspects of biology—organisms and parts of organisms have become adapted in the past so that they perform particular functions now. How can one make such systems philosophically respectable?

The main purpose of this book is to discuss the methods available to the morphologists in their attempts to determine the function of structures. Dullemeijer spends much time discussing the problem of how one can make useful statements about the functions of structures, while acknowledging the truism that the functions can in no way cause the existence of the structures, as they have evolved *pari passu*. The author suggests there are only three methods of much use: the experimental method; the paradigm method, when crudely we think how we would do something and then see whether nature makes as good a job of it; and the comparative method, when we examine structures performing, possibly, the same function.

This is a difficult book. The level of abstraction is high at the beginning and end; in the middle the text becomes a detailed and somewhat controversial review of bone and bony skeletons. In general this middle section would be quite interesting to someone interested in skeletons, though there are a great number of statements with which one might disagree. But, if one is illustrating a principle, surely it would be better to keep to less controversial material. It is too technical to be accessible to the average morphologist. What is he or she to make of this, for instance: "On the whole, the resistance to mechanical influence varies in the spongiosa from maximal (the trabeculae) to zero (the interspace). This, at first sight self-evident, heterogeneity has an interesting consequence, because in calculations of bony structure there are specific marginal effects that have to be taken into account. Such effects play a very minor effect in homogenous material. Some hold the view that the elasticity modulus for spongiosa should be smaller than for compacta. Considering the above-mentioned marginal effects, which will affect measurements, this seems at least doubtful."

That passage, apart from airing but not resolving a rather technical point, shows two other characteristics of this section. First, most workers on bone

would disagree with it: the modulus of elasticity of the spongiosa is much lower than that of the compacta; that is a well attested fact. Second, the quality of the English is such that one is never quite sure one has grasped the author's meaning. Unfortunately, this fault is found throughout the book. The phrase "specific marginal effect" for instance, is crucial to the sense of the quoted paragraph, but its meaning is quite obscure. In such a philosophical book this persistent lack of clarity in exposition is most damaging to sense and, after a while, to the reader's interest.

If morphologists are worried about the philosophical foundations of their work and if, unless experts, they are prepared to take the middle section on trust (which they should not), then this book may perhaps make them feel more or less comfortable. It is unlikely, though, that it will have much effect on how they do their science.

J. D. Currey

Nucleic acids

Basic Principles in Nucleic Acid Chemistry. Edited by Paul O. P. Ts'o. Vol. 1: pp. xii+636; vol. 2: pp. xi+519. (Academic: New York and London, April and June 1974.) Vol. 1: £18.00, \$37.50; vol 2: £16.55, \$34.50.

AMONG multi-author books, these two volumes are unusual in that the editor has also written three long chapters which together form 37% of the text. His first chapter ("In the Beginning") ranges far beyond the title of the series, from a historical review of nucleic acid research to prebiotic evolution, to contemporary biochemistry and, finally, to the destiny of man. The range is so wide that it is not surprising to find occasional errors, such as the statement that cyclic AMP transmits the effects of estrogen. There are also a number of spelling mistakes, and some of them also appear in the index. So many facts are mentioned that a bewildered reader might be forgiven for his relief at reaching the "Concluding remarks on Evolution" (page 69). The few paragraphs in this section are, however, very confused indeed and they do not conclude the discussion of evolution. "How", "why" and "fathomless" are used imprecisely, the artificial creation of living forms is irrelevantly introduced, and the difficulty of knowing the exact route of prebiotic evolution is wrongly equated with the problem of understanding gravity.

In the final section of the chapter (The Beginning of the Influence of Nucleic Acid Research on the Future of Man — "Intelligent" Evolution), Ts'o argues that human nature should be altered by genetic development. He states dramatically "our present genome . . . carries the cause of our impending

ing destruction and has become inadequate for the continuing evolution of man. We must develop a new species to succeed *Homo sapiens sapiens*." Skinner's stratagem to apply behavioural science to avert the end of man is considered to be futile ("How can a controlled society be designed by behaviourists in which unknown change of man is permitted and encouraged?"). To spice the mixture of arguments, there are genuflections to Teilhard de Chardin and overtures to ESP. Yet Ts'o never asks how we can hope to determine exactly which genetic changes are needed in order to alter human behaviour predictably.

The other chapters are invaluable advanced reviews by authors who have each made leading contributions to their topics. Some, such as Eisenberg and Bauer and Vinograd, have succeeded in being critical and selective, but the general tone is encyclopaedic. Thus, the volumes are more likely to be used as a treasure house of reference data than as teaching material, except in very specialised postgraduate work. In this respect the title of the series is misleading. Volume 1 has a comprehensive chapter on chemical synthesis and transformations of nucleosides (L. Goodman), followed by clear, stimulating accounts of mass spectrometry (McCloskey) and excited states (Gueron, Eisinger and Lamola). Tsuboi's chapter on infrared and Raman spectroscopy assumes a prior knowledge of Raman spectroscopy and the

volume ends with a long chapter by the editor on the structure of bases, nucleosides and nucleotides and their interactions with each other and with polymers.

Volume 2 is of more general interest to biochemists. It has excellent chapters on the chemical reactions of polynucleotides and nucleic acids (D. M. Brown), ultraviolet spectroscopy, circular dichroism and optical rotatory dispersion (Bush), hydrodynamic and thermodynamic studies (Eisenberg) and on circular DNA (Bauer and Vinograd). A final chapter by Ts'o is on the conformations and interactions of dinucleotides and oligonucleotides. The two chapters by Ts'o at the end of each volume are long and very detailed indeed, but they are in a field in which he has made important and extensive contributions. Naturally, there is some overlap with other chapters but it is often useful to discuss the material in a different context.

Although the preface remarks on the one hundred years since Miescher, it is more to the point that nineteen years have passed since the publication of the first two volumes of *The Nucleic Acids* edited by Chargaff and Davidson. Despite the presence of irrelevant material, the present volumes are particularly welcome, since the physicochemical aspects of nucleic acids have been reviewed far less than their biology and enzymology. We can look forward to the later volumes of the series.

K. Burton

All about the gibbon and the siamang

Gibbon and Siamang. Vol. 3: Natural History, Social Behaviour, Reproduction, Vocalization, Prehension. Edit. by Duane M. Rumbaugh. Pp. vii+208. (Karger: Basel and London, 1974.) SFr.130; £18.85; \$40.30; DM124.

This volume contains one long paper by Ellefson, reporting a field study on the white-handed gibbon *Hylobates lar*, and three much shorter papers. These are, respectively, an account by Brockelman, Ross and Pantuwatana of the social behaviour of a small experimental colony of *H. lar* on an island in the Gulf of Thailand, a description in terms of functional morphology of the hylobatid hand by R. Lorenz and an analysis of vocalisations from captive gibbons and siamangs by Tembrock.

Unfortunately, much of the value arising from the fact that the papers are published together in the one book, rather than appearing separately in journals, is lost by the fact that there is no liaison between them. For example, when Brockelman *et al.* and Lorenz refer to Ellefson's work they refer to his unpublished doctoral dissertation rather than to his paper in this book, and Tembrock's paper contains

no reference to Ellefson's work at all. This lack of liaison may have been no fault of the authors, but it does point to poor planning in the production of the book.

The three short papers are each useful additions to the primate literature. Ellefson's paper is more of a problem. The blurb on the dust jacket says "Observational data have been reduced when appropriate to quantitative summaries, but their interpretation and/or possible meaning is never lost." This policy seems little short of disastrous. It ensures that no reader can check the validity of Ellefson's conclusions against his data, nor does it allow detailed quantitative comparisons with published data from other primate field studies. In addition to the cavalier treatment of data, the treatment of theoretical issues, such as the aetiology of vocalisations and the concept of dominance, are naive in comparison with the relatively sophisticated literature that has been published on these topics in recent years. It is disappointing that what was clearly such a diligent study should have been so poorly reported.

N. R. Chalmers



Spectrophysics

LADY ANNE THORNE

Hardback: September 1974: 416 pages: 163 line illustrations: 412 12510 2: £6.50
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Spectrophysics describes the main methods of experimental spectroscopy and their application to the study of physical phenomena, with particular reference to the determination of transition probabilities and to problems in laboratory and astrophysical plasmas.

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The seventh edition of this readable introductory account of the physiology of insects has been completely updated and includes a new chapter on insect hormones. This book will be intelligible to any reader with no more than an elementary knowledge of biology but at the same time it gives a very complete survey of the subject. References are given to more complete treatment where necessary.

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Introduction to Optimization Methods

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Limp Binding: October 1974: 214 pages: 412 11040 7: £2.50

This book is an introduction to non-linear methods of optimization. The basic methods of single and multi-parameter optimization, presented here in a simple uniform terminology, are an essential prerequisite to a study of the current advanced methods reviewed under a few generic headings in the final two chapters.

Further information on these titles and a list of stockists is available from the publishers on request.

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announcements

Appointment

Desmond Gareth Julian has been appointed to the British Heart Foundation Chair of Cardiology at the University of Newcastle-upon-Tyne.

International meetings

December 3–5, **European Conference on Irradiation Behaviour of Fuel Cladding and Core Component Materials**, Germany, (Dr D. R. Harries, Metallurgy Division, AERE, Harwell, Didcot, Berkshire, UK).

December 5, **The Analysis of Gaseous Effluents**, Teesside (The Society for Analytical Chemistry, Analytical Division, Chemical Society, 9/10 Savile Row, London W1X 1AF).

December 5–6, **Discussion Meeting on the Theory of Electric and Magnetic Waves in the Ionosphere and Magnetosphere**, London (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG).

December 9–13, **Symposium of the International Atomic Energy Agency on Ionizing Radiation for the Sterilisation of Biomedical Products and Biological Tissue**, Bombay (International Atomic Energy Agency, PO Box 500, A-1011, Vienna, Austria).

December 9–14, **65th Exposition de Physique**, Paris (Société Française de Physique, Comité d'exposition, 33 rue D'Exposition, 75013 Paris).

December 12, **Membrane Components Involved in Transport and Diffusion**, Oxford (Dept. of Biochemistry, University of Oxford, Oxford OX1 3QU).

December 15–20, **1st Asian Regional Congress on Radiation Protection**, Bombay (Mr S. D. Soman, Chairman, Organising Committee RCRP, Modular Laboratories, Room 1-216H, Bhabha Atomic Research Centre, Bombay 400-085, India).

December 29, **2nd International Congress of Biorheology**, Israel (Dr F. A. Meyer, Weizmann Institute of Science, Rehovot, Israel).

Corrigenda

In the article "Expansion pattern of flare-associated disturbances near the

Earth's orbit during October 23 to November 4, 1968" by K. Sakurai (*Nature phys. Sci.*, **246**, 70; 1973) there were two errors in Table 1. The first number in column 1 should read 24 not 23, and the second number in the last column should read 79.5 not 69.5.

In the article "Effect of foetal calf serum and epidermal growth factor on DNA synthesis in explants of chick embryo epidermis" by S. Bertsch and F. Marks (*Nature*, **251**, 517; 1974) the first sentence of the legend to Fig. 1 should be replaced by, "Effect of epidermal growth factor (EGF) on the pulse-labelling of DNA in chick embryo epidermal explants precultivated for 24 h in the absence (a) or presence (b) of serum. After precultivation, the medium was replaced by serum-free medium containing EGF and the incubation was continued for the time indicated." In the last part of the legend omit the sentence, "The explants were preincubated in MEM (without serum) for 24 h before the addition of EGF."

Erratum

In the article "Grassland species can influence the abundance of microbes on each others roots" by P. Christie, E. I. Newman and R. Campbell (*Nature*, **250**, 570; 1974) the legend to Table 2 should read, *In part a letters (a and b) are for comparisons among the four weights, in part b for comparisons within each row.

Reports and Publications

Great Britain

- Already Too Many. (An Oxfam Special Report on Population, Family Planning and Development.) By Bernard Llewellyn. Pp. 20. (Oxford: Oxfam, 1974.) [128]
- Bulletin of the British Museum (Natural History). Zoology, Supplement 6: The Cichlid Fishes of Lake Victoria, East Africa—The Biology and Evolution of a Species Flock. By P. H. Greenwood. Pp. 1. 134+1 plate. (London: British Museum (Natural History), 1974.) £3.75. [138]
- Proceedings of the Royal Society of London. A: Mathematical and Physical Sciences, Vol. 339, No. 1618: A Discussion on the Exploitation of British Mineral Resources (other than Coal and Hydrocarbons) in Relation to Countryside Conservation. Pp. 271–416. (London: The Royal Society, 1974.) £2.75. [148]
- Bulletin of the British Museum (Natural History). Entomology, Vol. 31, No. 1: The British Cynipoidea (Hymenoptera) Described by P. Cameron. By J. Quinlan. Pp. 1–21. £1.15. Supplement 23: A Revision of the Leucospidae (Hymenoptera: Chalcidoidea) of the World. By Z. Bouček. Pp. 241. £11.30. (London: British Museum (Natural History), 1974.) [148]
- The Techniques of Analytical Chemistry: a Short Historical Survey. By Dr. H. M. N. Irving. (A Science Museum Survey.) Pp. 35. (London: HMSO, 1974.) £1 net. [158]
- The Use of Radio Frequencies for Sound and Television Broadcasting in the United Kingdom. (A paper by the BBC for its General Advisory Council.) Pp. 12. (London: BBC, 1974.) [158]
- Mineral Resources Consultative Committee. Mineral Dossier No. 8: Sulphur. Compiled by A. J. G. Northolt. Pp. vi + 46. (London: HMSO, 1974.) 47p net. [158]
- Plant Breeding Institute. Annual Report 1973. Pp. 191. (Cambridge: Plant Breeding Institute, 1974.) £1.50. [168]
- National Institute of Industrial Psychology. Annual Report and Statement of Accounts for the year ended September 30, 1973. Pp. 61. (London: National Institute of Industrial Psychology, c/o North East London Polytechnic, Livingstone Road, E15, 1974.) [168]
- Agricultural Research Council. Institute of Animal Physiology, Babraham, Cambridge—Report for 1972–73. (7th Biennial Report). Pp. 126. (London: Agricultural Research Council, 1974. Obtainable from HMSO.) £1.30 net. [168]

- Select Committee on Science and Technology. Government Observations on Third Report, Session 1972–73—Tracked Hovercraft. Ltd. Pp. 11. (Cmd. 5711). (London: HMSO, 1974.) 11p net. [198]
 - British Aid and the Relief of Malnutrition—Report of the ODA Advisory Committee on Protein. Pp. 30. (London: Ministry of Overseas Development, 1974.) [198]
 - Bulletin of the British Museum (Natural History). Zoology, Vol. 27, No. 2: Miscellanea. Pp. 49–138 + 7 plates. (London: British Museum (Natural History), 1974.) £4.70. [208]
 - National Radiological Protection Board. NRPB R23: The Study of Chromosome Aberration Yield in Human Lymphocytes as an Indicator of Radiation Dose. IV: A Review of Cases Investigated—1973. By R. J. Purrott, D. C. Lloyd, J. S. Prosser, G. W. Dolphin, Elaine J. Eltham, Patricia A. Tipper, Carolyn M. White and Susan J. Cooper. Pp. 27. (Harwell, Didcot: National Radiological Protection Board, 1974.) [208]
 - Journal of Biogeography, Vol. 1, No. 1, March 1974. Edited by David Watts. Pp. 1–74. Published Quarterly. Annual subscription £10 (USA and Canada \$34.) (Oxford: Blackwell Scientific Publications, Ltd., 1974.) [228]
 - British Antarctic Survey. Scientific Reports, No. 79: The Dynamics of the Brunt Ice Shelf, Coats Land, Antarctica. By R. H. Thomas. Pp. 45 + 3 plates. (London: British Antarctic Survey, Natural Environment Research Council, 1973.) £2.75 net. [218]
- ### Other Countries
- CERN—European Organisation for Nuclear Research. Annual Report for 1973. Pp. 235. (Geneva: CERN, 1974.) [78]
 - United States Department of the Interior: Geological Survey. Professional Paper 804: Erosional and Depositional Aspects of Hurricane Camille in Virginia, 1969. By Garnett P. Williams and Harold P. Guy. Pp. vi + 80. (Washington, DC: Government Printing Office, 1973.) \$1.80. [78]
 - International Directory of Woody Plant Physiologists. Compiled by Gregory N. Brown and James R. Brande. (Columbia, Missouri: Forest Service, US Department of Agriculture, in co-operation with School of Forestry, Fisheries and Wildlife, University of Missouri, 1974.) [88]
 - International Atomic Energy Agency. Annual Report, July 1, 1973–June 30, 1974. Pp. 75. The Agency's Programme for 1975–80 and Budget for 1975. (Vienna: International Atomic Energy Agency, 1974.) [98]
 - Fondation Universitaire, Bruxelles. 53e Rapport Annuel. Pp. 95. (Bruxelles: Fondation Universitaire, 1974.) [128]
 - World Health Organization. Technical Report Series, No. 552: WHO Expert Committee on Tuberculosis—Ninth Report. Pp. 40. (Geneva: WHO, 1974.) [128]
 - FAO/WHO/OIE. Animal Health Yearbook 1973. Pp. 199. (Rome: FAO; London: HMSO, 1974.) £1.80; \$4.50. [128]
 - Publications du Centre National pour l'Exploitation des Océans (CNEXO). Fascicule 2, 1974: Recueil des Travaux du Centre Oceanologique de Bretagne (COB). Pp. 652. (Paris, Cedex: CNEXO, 1974.) [128]
 - Organisation for Economic Co-operation and Development. Mercury and the Environment: Studies of Mercury Use, Emission, Biological Impact and Control. Pp. 196. (Paris: OECD; London: HMSO, 1974.) 25 francs; £2.50; \$6.25. [158]
 - United States Department of the Interior: Geological Survey. Water-Supply Paper 2027: Analog Model Study of the Ground-Water Basin of the Upper Coachella Valley, California. By Stephen J. Tyley. Pp. v + 77. \$1.05. Professional Paper 387-B: Recent Activity of Glaciers of Mount Rainier, Washington. By Robert S. Sigafos and E. L. Hendricks. Pp. vi + 24. \$5.70. (Washington, DC: Government Printing Office, 1972 and 1974.) [198]
 - Contribuciones del Instituto Antártico Argentino. No. 107: Segunda Contribución al Conocimiento del Microplankton del Mar de Bellingshausen. Por Enrique Balech. Pp. 63. No. 142L: Distribución Vertical de la Fauna Benomica en Tres Localidades Antárticas—Bahía Esperanza, Isla Petermann y Archipiélago Melchior. Por N. B. Bellisio, R. B. Lopez y L.A.P. Tomo. Pp. 87. No. 152: Interpretación de las Pulsaciones Aurorales en Términos de Fenómenos Ondulatorios. Por H. A. Cazeneuve. Pp. 19. No. 155: Estudio Diatomológico del Mar de la Flota, de Puerto Paraíso y Observaciones en el Mar de Bellingshausen. Por L. J. C. Martínez Macchiavello. Pp. 103. No. 157: Biosíntesis de Esteroides por la Glandula Suprarrenal de la Foca de Weddell—un Ejemplo de Adaptación Evolutiva y Ecológica. Por M. A. Borrrel, P. Borrrel, M. C. Damasco y C. P. Lantos. No. 161: Notas Biológicas sobre la Isla Pedro I. Por L.A.P. Tomo. Pp. 26. No. 162: Geología del Sector Noroccidental de la Península Hurd, Isla Livingston, Shetland del Sur, Antártica Argentina. Por R. Caminos, et al. Pp. 32. No. 166: Estudio *in vitro* sobre el Mecanismo de Secreción de Insulina en el Pinguino Papúa (Pygoscelis Papua). Por J. M. S. Farina y R. A. Chieri. Pp. 17. (Buenos Aires: Dirección Nacional del Antártico, Instituto Antártico Argentino, 1972 y 1973.) [208]
 - New Zealand: National Roads Board. RRU Bulletin No. 17: Vehicle Occupancy, Trip Purpose, Traffic Composition and Operating Speed—Feasibility Study and Pilot Survey. Pp. 48. (Wellington, NZ: National Roads Board, Road Research Unit, 1973.) [208]